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STUDY OF THE POSTULATED IDENTITY OF *HOFERELLUS CYPRINI* (DOFLEIN, 1898) AND *MITRASPORA CYPRINI* FUJITA, 1912

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In the kidneys of two- and three-summer old common carp (*Cyprinus carpio* L.) obtained from pond farms the so-called *Hoferellus cyprini* nodes were found to occur mainly in the autumn, plasmodia regarded as *Mitraspora cyprini* in the winter, whereas spores early in the spring. It is concluded that the species *Mitraspora cyprini* Fujita is synonymous with *Hoferellus cyprini* Doflein. *H. cyprini* develops in a one-year cycle: from October its early vegetative stages are situated intracellularly in the epithelium of renal tubules, parasitize several neighbouring epithelial cells, and thus constitute a single parasitic focus. From December the parasites gradually get into the lumen of renal tubules, in which they develop into plasmodia. Spores may develop already in the tubules; however, in most cases spore formation takes place in the ureter or urinary bladder.

A redescription of spores of *Hoferellus cyprini*, whose validity was contested by several authors, has also been performed.

Keywords. *Hoferellus cyprini* (Doflein, 1898), *Mitraspora cyprini* Fujita, 1912, identity, common carp (*Cyprinus carpio* L.), spore, morphology, description, developmental cycle.

Hoferellus cyprini was described from common carp (*Cyprinus carpio* L.) by Doflein (1898), who found in the renal tubules of common carp amoeboids 20 to 30 μm in size and pyramidal, longitudinally striated spores having characteristic polar projections. The polar capsules lay in the sutural plane. Other *Hoferellus* spp. were found in *Carassius auratus gibelio* by Achmerov (1960) and in crucian carp *(Carassius carassius)* by Golikowa (1960); they described these as *H. carassii* and *H. schulmani*, respectively. The spores of these parasites resembled those of *H. cyprini*, with the difference that there were bristle-like filaments at their ends.

The development of *H. cyprini* was studied by Plehn (1924), who, in addition to plasmodia and spores parasitizing the lumen of the renal tubules, found the early stages developing in the form of intracellular foci in the epithelial cells of the renal tubules. She established that the parasite was characterized by a one-year developmental cycle which was completed by the end of winter.

In Japan, from the renal tubules of common carp and goldfish Fujita (1912) reported a myxosporean resembling *H. cyprini*. The parasite was identical in size with *H. cyprini*; however, at the ends of its spores there were bristle-

like filaments and the suture connecting the two hemispheres of the spores ran in the plane between the spore capsules. Fujita (1912) named this parasite *Mitraspora cyprini*.

Thorough investigations into the development and pathology of *M. cyprini* were made in Japan by Ahmed (1973*a* and *b*) who attributed the disease characterized by kidney enlargement in the fish to this parasite. During the one-year developmental cycle of the parasite, in the summer and autumn Ahmed (1973*b*) found intracellular stages in the epithelium of renal tubules, in the winter coelozoic plasmodia in the renal tubules, whereas in the spring spores in the lumen of the renal tubules.

After its description *H. cyprini* was not reported to occur in Europe over a long period, and its existence was indicated by curricular data only (Schäperclaus, 1954; Bauer et al., 1969). Its recurrence was reported only in 1981 by Lom and Dyková. It was also Lom and Dyková (1981) who demonstrated the first European occurrence of *M. cyprini*, in Czechoslovakia. Subsequently Körting and Hermanns (1984, 1985) demonstrated both *H. cyprini* and *M. cyprini* from ponds of Southern Saxony. Furthermore, from the illustrations made by Lom and Dyková (1981) it undoubtedly appeared that the formation described in Hungary by Molnár (1980) as a *Myxobolus* cyst was actually a *Hoferellus* stage.

In the present paper, by comparing data of the literature with results of our own investigations, we furnish evidence that *M. cyprini* is a synonym of *H. cyprini*.

Materials and methods

The test material consisted of two- and three-summer old common carp, originating from various pond farms and submitted in 1982 and 1983 to the Central Veterinary Institute for routine examinations, primarily for demonstrating *Sphaerospora renicola* infection.

In 1984 and 1985 our studies were restricted mainly to the Fish Farm of Lovászpatona, where three generations were kept together in a pond and where *Hoferellus* infection far exceeded the average in Hungary. To survey *Hoferellus* infection, two- and three-summer common carp from this pond were examined at two-week intervals in the autumn and spring, and at one-month intervals in the winter. In the summer only common carp fry were submitted to the laboratory. Three to ten fish were dissected on each occasion. The kidneys were removed in their entirety, in association with the ureter and urinary bladder, and one part of each organ was studied in fresh.

From all the kidney parts examined, impression smears were prepared and stained with Giemsa; deep-frozen sections were made from one fish on each

occasion. Kidney pieces of fish that had proved positive in the fresh examination were fixed in 10% formalin or Bouin's solution, embedded in paraffin, and sections 4 to 8 μm in thickness prepared from them were stained with haematoxylin and eosin and according to Farkas and Mallory.

Results

Our studies were prompted by two observations made during routine diagnostic work. Studying the development of *Sphaerospora renicola* and the infection caused by it, we regularly found the *Hoferellus* foci (Fig. 1) described by Plehn (1924) in the kidneys of two- and three-summer old common carp. At other times plasmodia of an unknown myxozoan (Fig. 2) were detected in the ureters; they proved to be stages of *Mitraspora cyprini* only after a thorough investigation and, particularly, after that spores had developed from them by the spring. *Hoferellus* foci were found to occur primarily in the autumn and early in the winter, while *Mitraspora* plasmodia in the winter and spring.

Systematic studies were conducted to clarify the relationships existing between these observations.

Hoferellus foci were demonstrable at earliest in fish examined in October. At that time foci described by Plehn (1924) were found in the kidneys of about one-quarter of the two- and three-summer fish submitted to the laboratory from different fish farms of Hungary, while in about two-thirds of those kept under regular control in the Fish Farm of Lovászpatona. These foci were 100–120×120–200 μm in size and spherical or elongated in shape; they were located along the renal tubule and imitated its shape. Histologically, intensive protozoan infection of the epithelium of renal tubules was established in certain circumscribed areas of the kidneys. The myxosporidian developmental stages, 6 to 8 μm in diameter and granular in structure, filled up and enlarged the epithelial cells and pushed the cells' nucleus towards the basement membrane. The borders of the cells were not distinguishable by light microscopy. In their cytoplasm the large parasitic mass got in the immediate vicinity of the lumen of renal tubules; however, it was still separated from the latter by a narrow cytoplasmic zone on which the brush border was well visible. Occasionally, part of the cells surrounding the lumen remained free from infection (Fig. 3).

In December, in a part of the foci the tubular epithelium showed discontinuities, and *Hoferellus* stages 6 to 8 μm in diameter appeared in the lumen of the tubules. From December onwards *Hoferellus* foci in the kidney gradually decreased in number, and in March such foci were demonstrable only exceptionally.

At the time when the number of *Hoferellus* foci started to decrease (in December and occasionally already at the end of November) oval or amorphous

myxosporean plasmodia 15–40×15–30 μm in size occurred in the ureter; later on they gradually increased in number. These plasmodia were found freely in the lumen or, occasionally, attached loosely to the wall of the ureter. In intensive infection the plasmodia filled part of the renal tubules as well (Fig. 4). In March and April the plasmodia got in more and more distal portions of the ureter and spores developed within them. Spore formation took place primarily in the ureter and urinary bladder; however, in intensive infection numerous spores were found also in the convoluted tubules. Three to ten spores were formed within each plasmodium. In fresh preparations the spores, 9×6.6 μm in size on the average, had the shape described by Hofer (1898), as shown in Fig. 5; however, a more thorough examination demonstrated the presence of caudal filaments and striae on them. The suture, which was hard to visualize, was found to run in the plane between the two spore capsules.

Based upon the developmental stages examined and the study of 25 spores, we give a redescription of the parasite as follows.

Hoferellus cyprini (Doflein, 1898)

The vegetative stages fall into two types. The early developmental stages are spherical trophozoites 6 to 8 μm in diameter (Fig. 6); they develop intracellularly in the epithelium of the renal tubules and contain 7 to 8 daughter cells (Fig. 7). These stages develop in a given portion of the tubules and from spherical or oval foci 100–200×120–200 μm in size in the kidneys. The late vegetative stages can be found in the lumina of renal tubules and ureters: these are transparent plasmodia of irregular shape and 15–40×15–30 μm in size. The endoplasm of plasmodia has granular structure and contains large refractile droplets. The plasmodia are polysporoblastic and 3 to 10 spores are formed within them. The mature spores (Fig. 8) are short ellipsoidal, 8.5 to 10 (9.0) μm long, 5.2 to 7.1 (6.6) μm wide and 5.2 to 5.8 (5.6) μm thick. Their anterior end is rounded and the posterior one is flattened. The polar capsules lay in the sutural plane. The suture only slightly protrudes over the surface of the spore and is hard to observe. The surface of the spore shell is furrowed; on it run 20 longitudinal striations which start from the apex of the anterior end and, slightly deviating from the spore shell, form a characteristic rim around it. As a continuation of the ribs, twenty bristles 4.5 to 5 μm in length project caudally from the spore. The spores contain two equal-sized polar capsules 3.5 to 4.2 (3.8) μm in length and 2.1 to 2.2 (2.15) μm in width; their anterior end is tapered, and the polar filament has four or five convolutions in them.

These spores, identified by us with spores of *H. cyprini*, essentially correspond to those described by Fujita (1912) and Kudo (1920) as *M. cyprini*, and differ from those illustrated by Ahmed (1973*a*) only in their somewhat "stubbier" conformation.

Discussion

From the present studies it may be concluded that the species descriptions of Doflein (1898) and Fujita (1912) cloak the same parasite; i.e. the species *Mitraspora cyprini* Fujita, 1912 is a synonym of *Hoferellus cyprini* (Doflein, 1898). Over a long period researchers were prevented from recognizing their identity because the species and genus description of Doflein (1898) was accepted by the subsequent investigators (Plehn, 1924; Achmerow, 1960; Golikowa, 1960; Shulman, 1966) practically without any revision. These investigators failed to notice that Doflein had illustrated the course of sutures of *H. cyprini* spores incorrectly. Doflein (1898) must have imagined the hardly-observable sutures of *H. cyprini* to resemble those of *Myxobolus* species, with which he was very familiar. Plehn (1924) studied mainly the development of *H. cyprini* and only little did she deal with its spore morphology. On the other hand, Golikowa (1960) and Achmerov (1960) noticed that there were long filaments at the ends of *Hoferellus* spores; however, they, too, illustrated the plane of sutures erroneously. At the same time, Achmerov (1960) was right reporting that at the posterior half of the approximately oval spores there was a ring-shaped formation.

Fujita's (1912) representation of *Mitraspora cyprini* reported by him from common carp and goldfish was essentially correct, and his description was accepted by Kudo (1920) and Ahmed (1973*a*).

With regard to the fact that, despite the erroneous representation, Doflein's (1898) description enjoys priority, the species described and the genus created by Fujita (1912) should be considered synonyms of the species *Hoferellus cyprini* and the genus *Hoferellus* Berg, 1898, respectively.

In addition to the morphological similarity of the spores, also the developmental cycle and the intracellular stages are strongly suggestive of the identity of *M. cyprini* with *H. cyprini*. Both Plehn (1924) and Ahmed (1973*a*) found that the parasite followed a one-year cycle, its early developmental stages appeared in the autumn, the plasmodia in the winter, while their spores in the renal tubules appeared in the spring. No difference exists between *M. cyprini* and *H. cyprini* in the morphology of the vegetative stages either, since the renal tubule lesions reported from goldfish by Ahmed (1973*a*) and Hoffmann (1984) as mitrasporosis were identical with those reported by Plehn (1924), Lom and Dyková (1981) and Körting (1984), and also with our findings.

The development of *H. cyprini*, as revealed by the present studies, is essentially consistent with that suggested by Ahmed (1973*a*), with the difference that we, due to the low incidence and intensity of infection, had no opportunity to study the early developmental stages occurring in the summer. The developmental stages found by us correspond to those described by Plehn (1924); however, Plehn (1924) assumed that the *Hoferellus* organisms released from the

epithelium of the renal tubules into the lumen formed spores immediately. Contrarily, the present findings indicate that the *Hoferellus* developmental stages released into the lumen first form a multinucleated plasmodium there, and spores appear only after a developmental period of some weeks or months.

Our observations contradict the hypothesis of Dyková et al. (1983) suggesting that intracellular *Hoferellus* stages are some other myxozoan developmental stages that have got to a deadlock of development. We can imagine that after the disruption of the foci some of these stages get into the interstices and are destroyed there; however, most of the developmental stages undoubtedly get into the lumen and form mature spores. Furthermore, we presume that the intracellular stages do not always form large, cyst-like foci; sometimes they infect solitary cells as well. Such parasites, considered by Lom and Dyková (1981) to represent *Sphaerospora renicola* developmental stages, can also be identified, in all probability, with *H. cyprini*.

Shulman (1966) considered the genus *Mitraspora* to be a synonym of the genus *Sphaerospora* Thelohan, 1982; thus, the identity of the genus *Hoferellus* with sphaerospores might be suggested. However, such an identity is excluded by the considerable development-biological differences existing between the two groups of parasites, despite their possible morphological resemblances.

Further studies are needed to clarify whether the very same *Hoferellus* species parasitizes the common carp, goldfish, crucian carp and *Carassius auratus gibelio*, and whether the species described by Achmerov (1960) and Golikowa (1960) should be regarded as synonyms of *H. cyprini* or as one or more distinct species.

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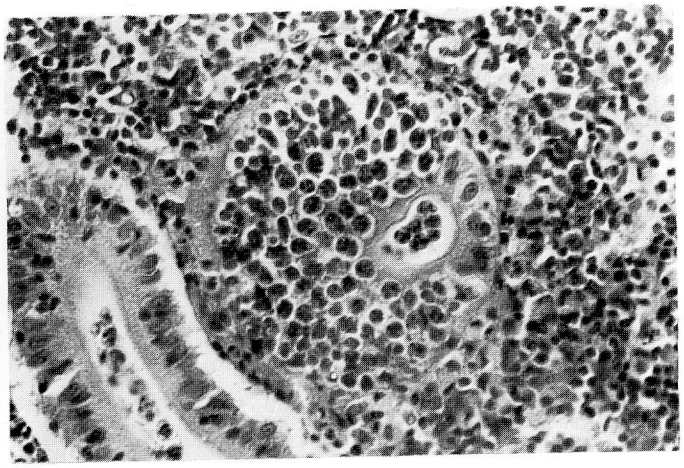

Fig. 1. Hoferellus "nodule" around the renal tubule in common carp. *Hoferellus* developmental stages are situated within the epithelial cells. In the lumen of the renal tubules developmental stages of *Sphaerospora renicola*, occurring as a frequent concomitant parasite, can be seen. Haematoxylin and eosin (H–E.), × 500

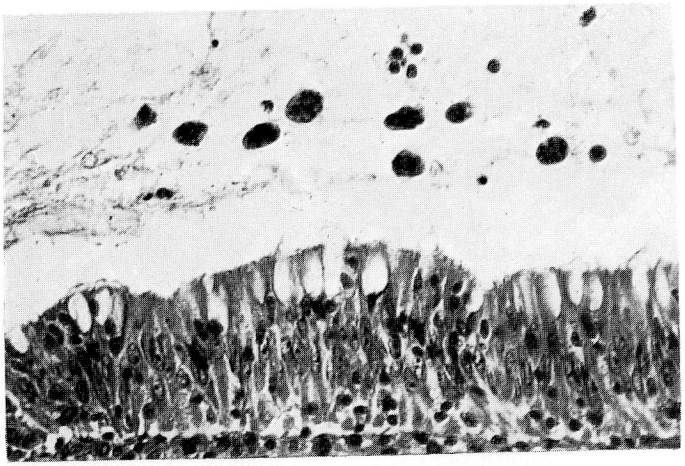

Fig. 2. Hoferellus plasmodia in the lumen of the ureter. H–E., × 600

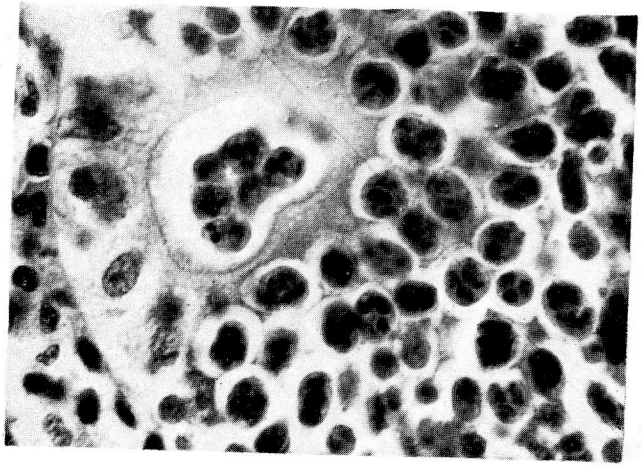

Fig. 3. Magnification of a part of Fig. 1. Intracellular *Hoferellus cyprini* trophozoites exceed in size the sphaerospores occurring in the lumen. On one side of the tubule there still are intact epithelial cells. *Hoferellus* trophozoites filling the infected epithelial cells are separated from the lumen of the tubule by a narrow cytoplasm. H–E., × 1200

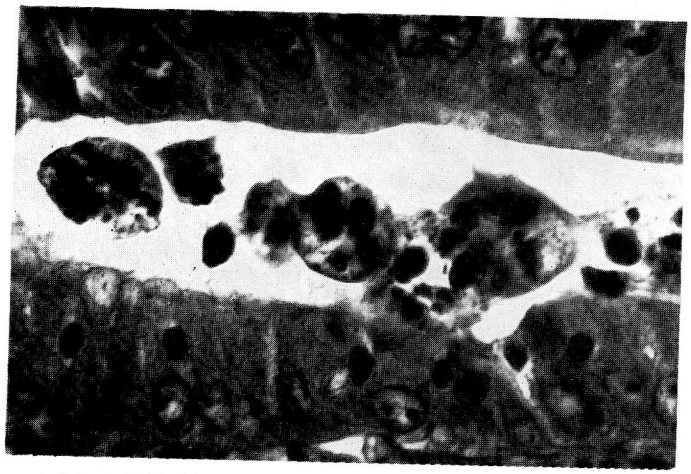

Fig. 4. Spore-containing *Hoferellus* plasmodia in the lumen of a renal tubule. H–E., × 1800

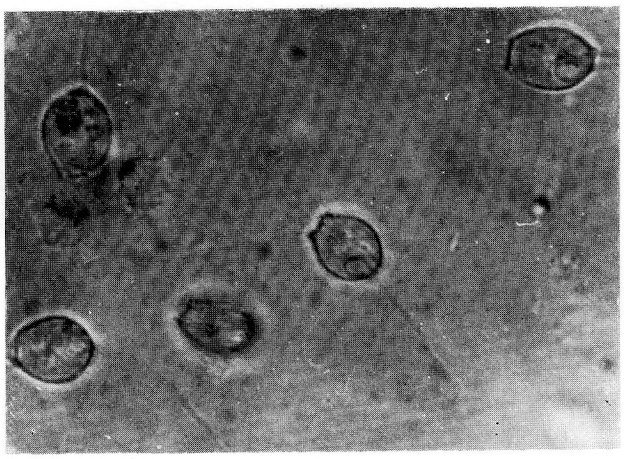

Fig. 5. Hoferellus cyprini spores. In part of the spores the filaments have been ejected from the polar capsule. Fresh preparation, × 2000

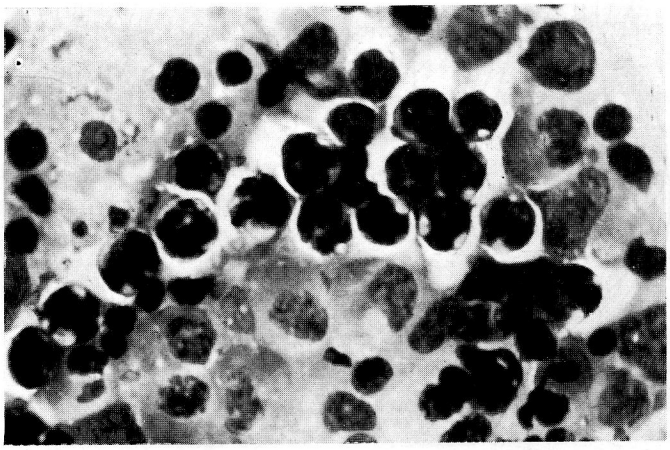

Fig. 6. Spherical trophozoites obtained from a *Hoferellus* nodule. Impression smear stained with Giemsa, × 1800

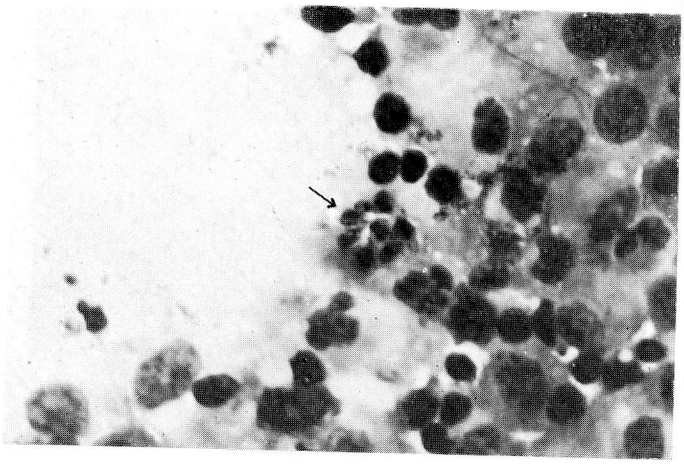

Fig. 7. Squashed *Hoferellus* trophozoite (arrow), consisting of secondary formations. Impression smear stained with Giemsa, × 1800

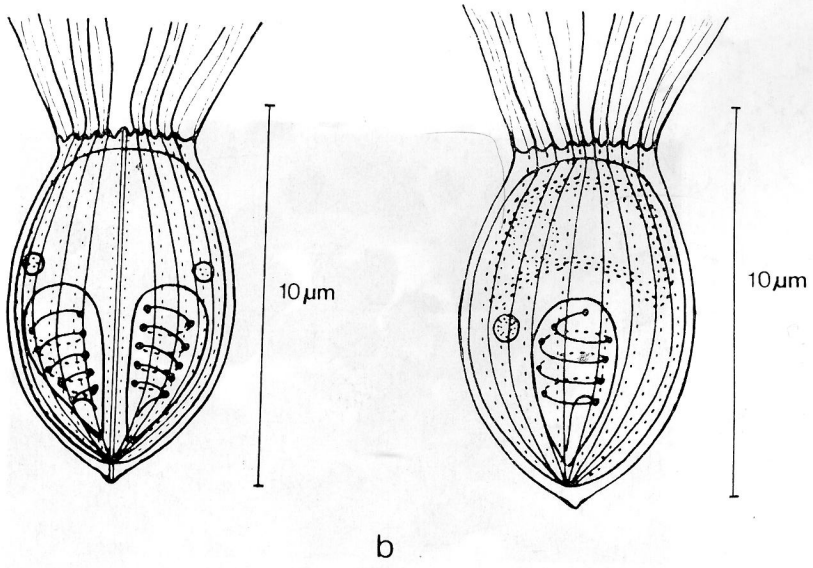

Fig. 8. Schematic presentation of *Hoferellus cyprini* spores. *a:* spore in frontal view; *b:* spore in transversal view