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HOFERELLUS CYPRINI (DOFLEIN, 1898) BERG, 1898 (SYN. MITRASPORA CYPRINI FUJITA, 1912), MYXOBILATUS NOSTALGICUS SP. N. AND RELATED SPECIES: PARTIAL REVISION OF TWO MYXOSPOREAN GENERA

J. LOM

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. Reasons are presented to consider the taxon *Hoferellus* Berg, 1898 a valid myxosporean genus while *Mitraspora* Fujita, 1912 is reduced to synonymy. Populations of *Hoferellus cyprini* (Doflein, 1898) Berg, 1898 from common carp (*Cyprinus carpio*) are differentiated from those infecting the goldfish (*Carassius auratus*) for which the name *H. carassii* Akhmerov, 1960 has to be reserved. *H. conifer* Gavrilova, 1966 and *H. schulmani* Golikova, 1960 have to be taken for junior synonyms of *H. carassii*. A new species of the genus *Myxobilatus*, *M. nostalgicus* sp. n. has been found to infect the renal tubules of tench (*Tinca tinca*) in Czechoslovakia. The spores have oviform body, 11 × 8.1 µm in size, with slightly curved, 8.5 µm long caudal processes. *M. gobii* Evlanov, 1981 from the renal tubules of gudgeon (*Gobio gobio*) has been redescribed. Two morphologically different populations of *M. legeri* (Cépède, 1905) Davis, 1944 have been found in the roach (*Rutilus rutilus*) and the rudd (*Scardinius erythrophtalmus*), respectively. Their taxonomic position is discussed.

Recently the interest in kidney infecting myxosporeans in freshwater fish has increased in view of the considerable pathogenicity of some of the species and because of their puzzling life cycles (e.g. Molnár 1980, 1984; Dyková and Lom 1982; Lom et al. 1983a; Clifton-Hadley et al. 1984; Kent and Hedrick 1985). Nevertheless, our understanding of these parasites is still rather unsufficient which applies especially to the genera *Mitraspora*, *Hoferellus* and *Myxobilatus*.

Mitraspora cyprini Fujita, 1912 was described from carp and goldfish in Japan and later found to be a serious pathogen of goldfish in the Far East and the U.S.A. It is the agent of the kidney enlargement disease (KED) as described by Ahmed (1974) and Hoffman (1981) who called this species a "kidney bloater" due to the swelling of the body cavity of the diseased fish. The genus *Mitraspora* was first reported from Europe by Lom and Dyková (1981) who found that a myxosporean corresponding to the description of Fujita (1912) was a common parasite of carp fingerlings in Czechoslovakia. The extensity of infection was sometimes up to 32 % (Lom et al. 1983b). It caused pathogenic lesions in the kidney.

The other species common in the renal tubules of carp in Czechoslovakia as well as in neighbouring countries is *Sphaerospora renicola* Dyková et Lom, 1982. Extensive surveys of carp kidney myxosporeans, however, failed to reveal mature spores of *Hoferellus cyprini*, a species described by Doflein (1898) in carps from the territory which is now Czechoslovakia under the name *Hoferia*, which name was emended by Berg (1898) to *Hoferellus*. According to Doflein (1898), the sutural line of the two shell valves coincides with the plane of the two polar capsules (as it does in *Myxobolus*). On the contrary, the sutural line found by Lom and Dyková (1981) in spores of what they determined as *Mitraspora* runs perpendicularly to the plane of the polar capsules (as it does in *Sphaerospora*). The arrangement of the sutural line in the relation to the polar capsules is one of the important characters of myxosporean spores which determine the classification of this group. More recently, myxosporeans which according to the spore structure have to be classified as *Mitraspora cyprini* were found in the Federal Republic of Germany (Körting and Hermanns 1985) and in Hungary (Molnár et al. 1986).

Proper identity and nomenclature of this myxosporean is of considerable importance in view of its pathogenicity. Doflein (1898), an eminent protozoologist, described *Hoferellus* from fresh and stained material. He recorded coelozoic vegetative stages of which he supplied a rather primitive drawing showing no caudal bristle-like appendages. He mentioned, however, that sometimes such "protoplasmic projections" can be seen. Mercier (1908) who studied this species on stained preparations did not mention the caudal bristles. This simple image of *Hoferellus* as of a species inhabiting the lumen of renal tubules where it produces spores in small trophozoites was complicated by Plehn (1924). She found cyst-like swellings of the epithelium of renal tubules packed full with globular bodies which she took for initial stages of vegetative reproduction of *Hoferellus cyprini*. She supposed that later — in late winter and early spring — these stages escaped into the lumen of the tubules where they started the production of spores. She did not describe the spores.

Consequently, there have been two problems; did the miter-shaped, bristled spores belong to the genus *Mitraspora* or *Hoferellus* and, second, did the intracellular stages in hypertrophic epithelial cells of the renal tubules really represent stages which later produce *Hoferellus*-spores?

The reasons, why these bodies cannot be taken for vegetative stages of *Hoferellus* but rather represent an abortive part of developmental cycle of another myxosporean, possibly *Sphaerospora renicola*, were explained in detail by Lom and Dyková (1985). First, ultrastructural examination has shown that the development of these bodies produces very complicated stages which eventually undergo spontaneous cell degradation making them obviously non-viable so that any further development seems highly improbable. Second, a year-round observation of the carp stock infected with the intracellular bodies failed to show any spore formation. Third, these infections were found in localities free from *Mitraspora* but infested with *S. renicola* (Grupcheva et al. 1985).

Since the miter-shaped spores failed to correspond to Doflein's original description, Lom and Dyková (1985) considered the genus *Hoferellus* as inadequately characterized and doubted its validity, unless spores with structure corresponding to Doflein's original description are found. The spores which Doflein (1898) and Mercier (1908) had actually seen could be the later described *Mitraspora cyprini*.

The species evidently enjoys a wide distribution in Czechoslovakia (Doflein 1898; Lom et al. 1983b), Germany (Körting and Hermanns 1985), Japan (Fujita 1912), France (Mercier 1908), and Hungary (Molnár et al. 1986). The probability of finding a spore which would correspond to Doflein's description is nil. For the future, two nomenclatural solutions are possible. One can adhere strictly to the fact that Doflein's description does not correspond to any existing organism and to supress the genus. The other is to admit, that he did not observe the fine sutural line correctly and had in fact described Fujita's (1912) organism. Then automatically *Mitraspora* becomes a junior synonym of *Hoferellus*. This position, first adopted by Molnár et al. (1986) is presently acceptable. That is why we in this paper deal with the miter-shaped spores under the name of *Hoferellus*.

Molnár et al. (1986), however, failed to supply thus far any convincing evidence for their claim that the intracellular bodies in the hypertrophic renal tubules really do represent part of the life cycle of *Hoferellus*.

The aim of the present paper is to compare the morphology of *H. cyprini* from carp and *H. carassii* from goldfish. We also compare morphology of three species of the genus *Myxobilatus* Davis, 1944, proposing to establish one of them as *M. nostalgicus* sp. n.

MATERIALS AND METHODS

The species described were found in the course of our long term survey of protozoan parasites of fish. Fresh mounts of kidney tissue and urinary tract were made. Drawings, measurements and photographs were taken of fresh material only. Additional supporting observations were made on histological sections of material fixed in 10 % formalin and stained with haematoxylin-eosin and Giemsa stains.

RESULTS

Hoferellus carassii Akhmerov, 1960

a) population from goldfish, *Carassius auratus auratus* L.
(Figs. 1A, and Plate I, 1 to 4)

Fresh spores were obtained from goldfish from the U.S.A. through the courtesy of Dr. Glenn L. Hoffman to whom our sincere thanks are due. The spores were miter-like, elongated, only rarely almost oviform, with a projecting anterior apex and a truncated posterior base. The length was 13 (11—15.2) μm, the width (in sutural view) 7.5 (6.2 to 9) μm, the thickness (in valvular view) 4.6 (4.2—6) μm which means that they were significantly flattened in the sutural plane. The surface of the spore shell bears a total of about 18—22 longitudinal ridges, which extend posteriorly, around the edge of the truncated spore bottom, into rigid bristles about 4.5—6 μm long. The drop-like polar capsules, 4.2 (3.3—5) × 2.4 (2—2.6) μm in size have 5 to 6 threads of the polar filament tightly coiled at an angle of about 45° to the longitudinal axis of the capsule. The anterior points of the capsules are set at a distance of at least 2 μm from the anterior tip of the spore.

b) population from feral *Carassius auratus gibelio* (Bloch)
(Figs. 1B, C and Pl. I, 5 to 8)

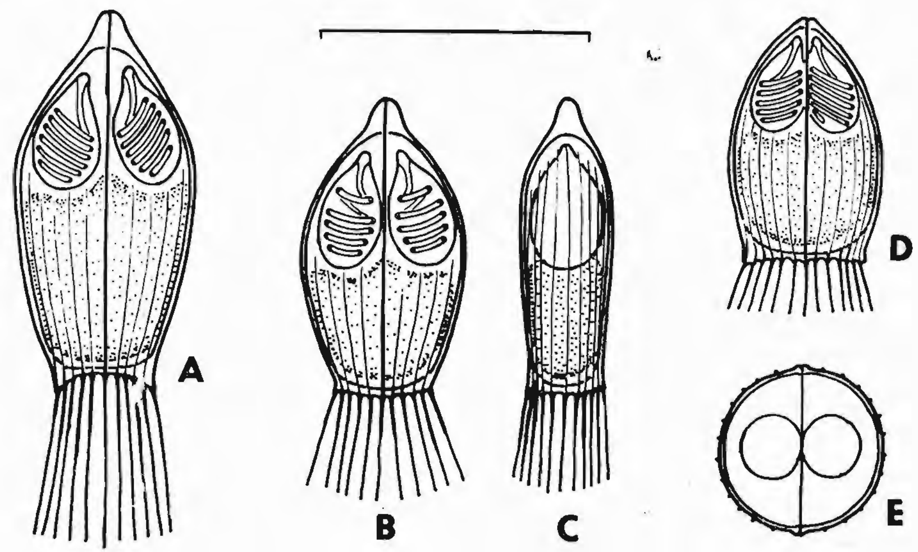

Fig. 1. A, B, C — *Hoferellus carassii*. A — typical spore from *Carassius auratus auratus* in sutural view. B — typical spore from *C. auratus gibelio* in sutural view; C — the same in a view perpendicular to the sutural plane. D — typical spore of *H. cyprini* in sutural view; E — optical cross section of the latter. Bar = 10 μm.

Found in renal tubules of hosts collected near Brzotín in the vicinity of Rožňava in East Slovakia in June 1984. Two out of 17 specimens examined were infected.

Plasmodial trophozoites up to 40 × 22 μm were observed to produce up to 4 spores each and contain a mass of refractile granules.

The spores are 10.6 (9.5—11.4) μm long, 6 (5—6.6) μm wide and 3.8 (3.6—4.5) μm thick and thus appear less elongated than in the preceding population. There is a total of 18 to 20 surface ridges. Polar capsules are 4.8 (4.4—5) × 2.8 (2.6—3) μm in size, there are 5 (4—6) threads of the polar filament coiled at an angle almost perpendicular to the longitudinal axis of the capsule.

In populations from both hosts, the number of spores measured (n) was 20.

Hoferellus cyprini (Doflein, 1898) Berg, 1898 (Figs. 1D, E; Pl. I, 9; Pl. II, 1 to 3)

Common in carp renal tubules, the extensity of infection being sometimes up to 32 % (Lom et al. 1983b).

Rounded or elongated plasmodia characterised by the presence of a large number of refractile granules can reach the size of 40 × 65 μm and produce, depending on their size, several to 10—12 spores.

The spores, more stubby than those of *H. carassii*, are bullet-like, the length is 9 (8—10.2) μm, width 6.5 (6—7) μm, thickness being about the same as the width. There is a total of 20—24 surface ridges and short, 2—4 μm long posterior bristle-like appendages. The polar capsules, 2.4 (2.3—2.5) × 3.6 (3.3—4) μm in size (n = 20) have threads of polar filament in the number of 5 tightly coiled at an angle of about 40° to the longitudinal axis of the capsule.

Myxobilatus nostalgicus **sp. n.** (Figs. 2A; Pl. II, 4 to 6)

In the renal tubules of *Tinca tinca* collected in the pond Vrbový at Brzotín near Rožňava, East Slovakia in June 1984. Found in one out of 10 specimens collected, in a mixed infection with *Sphaerospora galinae* Evlanov and the trophozoites could not be distinguished safely. That is why their description is not presented.

The body of the spore is regularly oviform, 11 (9.8—12.3) × 8.1 (7.7—8.8) μm in size (n = 13). The shell appeared smooth, no surface striation could be detected. At the anterior apex of the spore the shell is slightly thickened and flattened. Posteriorly, each shell valve extends into a slender, slightly curved caudal process, 8.5 (7—9.5) μm long. Unlike other *Myxobilatus* or *Henneguya* species, the processes are set apart at their bases and tend to cross over at their ends. Along their sutural line, there is no distinctly elevated ridge. Drop-like polar capsules, 4.2 (3.6—4.7 × 3 (2.7—3.3) μm in size have 5 to 6 tightly coiled threads of the polar filament situated at about 30° to the longitudinal axis of the capsule. Slides with the type specimens are deposited in the Institute of Parasitology, Czechoslovak Academy of Sciences.

Myxobilatus gobii Evlanov, 1981 (Fig 2B)

In the renal tubules of *Gobio gobio* collected in May 1983 in the pond drainage system at Klec near Třeboň, Czechoslovakia. Found in 1 out of 16 specimens collected at the locality.

Trophozoites the size of up to 35 μm have cytoplasm with small refractile granules and produce several spores.

The body of the spore is oviform, anteriorly tapered, 10.2 (9.5—11.4) × 6 (5.6 to 6.2) μm in size (n = 10). The shell valves bear fine longitudinal surface ridges (about 12 around the circumference of the spore) and posteriorly extend each into a long — 18 (11 to 19) μm — straight, gradually tapering caudal process. Drop-like polar capsules,

4.5 (4.2—5) × 2.2 (2—2.9) μm in size contain 6 to 7 threads of the polar filament tightly coiled at an angle of about 30° to the longitudinal axis of the capsule.

Myxobilatus legeri (Cépède, 1905) Davis, 1944

a) population from *Rutilus rutilus* (Fig. 2C)

In the renal tubules of roach collected in the pond drainage system at Klec near Třeboň, Czechoslovakia. Found in 3 out of 20 specimens examined.

Trophozoites that we have observed were up to only about 12 × 30 μm in size, filled with yellowish, refractile granules the size of up to 1 μm. They had an amoeboid shape with ectoplasmic region well differentiated and protruding into fine and branched pseudopodia. They contained one or two spores only.

The spore body was spindle-shaped, anteriorly pointed, 12 (11.5—13) × 5.2 (4.8 to 5.2) μm (n = 20) in size. There was a total of about 10 fine longitudinal surface ridges. The caudal projections were slender, gradually tapering to the end and were slightly curved and divergent from their base. Their length was 7 (6—9) μm. Elongated, anteriorly pointed polar capsules, 4 (3.8—4.2) × 2 (2.1—2.3) μm in size had 6 threads of tightly coiled polar filament set at an angle of about 40° to the longitudinal axis of the polar capsule.

b) population from *Scardinius erythrophtalmus* (L.) (Fig. 2D)

Found in 1 out of 4 rudds examined collected in the Lion branch of the Danube river near Klučovec, Slovakia, in July 1981.

Large agglomerations of trophozoites, up to 22 μm in size, considerably distended the tubules. There were just few mature spores, 13 × 5.5 μm in size and with caudal projections 4.4 μm long. The polar capsules measured 2.5 × 1.4 μm and had 5 threads of the polar filament. There was a total of 15 surface ridges.

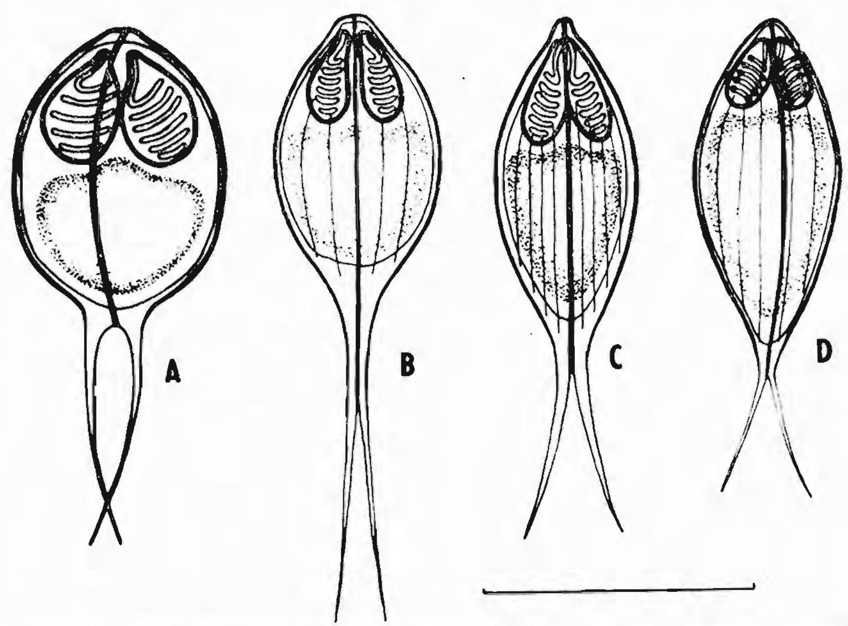

Fig. 2. Typical specimens of spores of A — *Myxobilatus nostalgicus;* B — *M. gobii;* C — *M. legeri* from *Rutilus rutilus;* D — *M. legeri* from *Scardinius erythrophtalmus.* Bar = 10 μm.

DISCUSSION

1. *Hoferellus*

Fujita (1912) and Hoshina (1968) made no difference between the populations from carps and goldfish. Shulman (1984) lists in his myxosporean key the species of *Hoferellus* both under the generic name *Sphaerospora* (taking *Mitraspora* for its junior synonym) and *Hoferellus*. As hosts of *S. cyprini* — in accord with Fujita (1912) — he quotes three hosts — *Cyprinus carpio haematopterus*, *Carassius auratus* (goldfish) and *Carassius auratus gibelio*, without differentiating the characters of *S. cyprini* from individual hosts and without stating which source does his illustration come from. In the genus *Hoferellus*, Shulman (1984) quotes four altogether different species.

Our material, however limited, suggests that there are significant differences in *Hoferellus* populations from *Cyprinus* and *Carassius*. Our observations on the spore shape of *Hoferellus cyprini* from carp essentially agree with other descriptions of populations from this host — Doflein (1898), Kudo (1919) and Molnár et al. (1986). Also the dimensions are similar (Kudo: 10 μm length, 8—9 μm width, 6—8 μm thickness; Molnár et al.: 8.5—10 μm length, 5.2—7.1 μm width, 5.2—5.8 μm thickness; only Doflein gave slightly greater length, 10—12 μm).

The dimensions and shape of spores that we recorded in cultured goldfish agree with those described from the same host by Ahmed (1973): length 11.2—14 μm, width 5.6—7 μm. The spores of *Hoferellus* populations from the feral *Carassius auratus gibelio* are quite similar to the spores from goldfish as opposed to *H. cyprini* from carp. The principal difference (see Fig. 1) that we could state is the snout-like anterior end of the spore, the polar capsules being not so close to the anterior spore tip, and a more elongated and more flattened spore in *Carassius* populations.

If we accept that populations from *Cyprinus* and *Carassius* are different species due to their morphology and host specificity* what should be the proper name for the latter? Three separate species have been described from *Carassius* (Shulman 1984):

Hoferellus carassii Akhmerov, 1960 from the renal tubules of *Carassius auratus* (goldfish?), length 12 μm. Found in the Amur river basin.

H. conifer Gavrilova, 1966 from the urinary bladder of *C. auratus gibelio*, length 8—13 μm. Found in Krym peninsula, in Volga and Syrdarja river basins.

H. schulmani Golikova, 1960 from the gall bladder (!) of *C. auratus* (goldfish?), length of spore 8—10 μm. Found in the Kaliningrad region of the USSR.

Which of the three possibly synonymous species should be selected to represent populations from *Carassius*? Since all the drawings of these species are rather diagrammatic and may not exactly correspond to the fine differences in spore shape, we select *H. carassii* Akhmerov, 1960 because of the date of description (as opposed to *H. conifer*) and site of infection (as opposed to *H. schulmani*).

Unless future studies show otherwise, we propose to consider the *hofferelli* from carp as *H. cyprini* and those from *Carassius* as *H. carassii*.

Shulman (1984) considered *Mitraspora* a junior synonym of *Sphaerospora* and in a sense this judgement of his might apply to the *Hoferellus* vs. *Sphaerospora* relation. We oppose this opinion because of spore differences which are of the same rank that he honours as generic character in other myxosporeans — no species of *Sphaerospora* has similar bristle-like appendages. Further, the trophozoites of the latter are pseudoplasmodia, in fact free mono- or disporous pansporoblasts, while in *Hoferellus* there is a large plasmodium with more vegetative nuclei and pansporoblast formation (Lom and Molnár, in preparation).

* In *Sphaerospora* species, with an identical site of infection, there seems to be a well pronounced host specificity. Different fish hosts seem to have their specific *Sphaerospora* species.

Since in this paper as well as in that by Molnár et al. (1986) the genus *Mitraspora* is considered a junior synonym of *Hoferellus*, a change has to be made in the recently proposed myxosporean classification (Lom and Noble 1984). *Hoferellus* has to be transferred from the family Myxobolidae, suborder Platysporina, to replace *Mitraspora* in the fam. Sphaerosporidae in the suborder Variisporina.

2. *Myxobilatus*

M. nostalgicus. This species clearly differs from all other twenty thus far recorded species of the genus by the broadly egg-shaped spore body and by the configuration of the caudal processes. Therefore we propose to establish it as a new species. Also, no *Myxobilatus* has thus far been recorded from tench.

M. gobii. Our findings obviously agree with Evlanov's (1981) record although he described the caudal processes to be much longer, up to 30 μm. Since Evlanov did not find the vegetative stages, did not describe the structure of the polar filament and his illustration is rather diagrammatic, we find it useful to redescribe the parasite.

M. legeri. Shulman (1984) quotes the rudd as host of this species. He lists additional seven species of cyprinid fish (of the genera *Abramis*, *Noemacheilus*, *Leuciscus*, *Blicca*) as the hosts. We find it preferable to present an exact description and comparison prior to suggestion of identity of species found in various hosts. Evaluation of a more abundant material may eventually prove the populations from rudd to be different from the type populations from the roach (e.g., conspicuously smaller polar capsules) and warrant establishing a separate species.

HOFERELLUS CYPRINI (DOFLEIN, 1898) BERG, 1898 (SYN. *MITRASPORA CYPRINI* FUJITA, 1912), *MYXOBILATUS NOSTALGICUS* N. SP. И РОДСТВЕННЫЕ ВИДЫ: ЧАСТИЧНАЯ РЕВИЗИЯ ДВУХ РОДОВ МИКСОСПОРИДИЙ

Й. Лом

Резюме. Приводятся доводы считать таксон *Hoferellus Berg*, 1898 действительным родом миксоспоридий и род *Mitraspora* Fujita, 1912 в качестве его синонима. Популяции *Hoferellus cyprini* (Doflein, 1898) Berg, 1898 от карпов (*Cyprinus carpio*) отличаются от тех, которые заражают золотых карасей (*Carassius auratus*) и для которых нужно оставить название *H. carassii* Akhmerov, 1960. *H. conifer* Gavrilova, 1966 и *H. schulmani* Golikova, 1960 являются синонимами *H. carassii*. Был найден новый вид рода *Myxobilatus* — *M. nostalgicus* n. sp., заражающий мочевые канальцы почек линя (*Tinca tinca*) в Чехословакии. Споры имеют овальное тело размером 11×8.1 мкм, со слегка изогнутыми хвостовыми отростками длиной 8.5 мкм. Повторно описан вид *M. gobii* Evlanov, 1981 из мочевых канальцев пескарей (*Gobio gobio*). Найдены две морфологически отличные популяции *M. legeri* (Cépède, 1905) Davis, 1944 соответстcovенно у плотвы (*Rutilus rutilus*) и у красноперки (*Scardinius erythrophtalmus*). Обсуждается их таксономическое положение.

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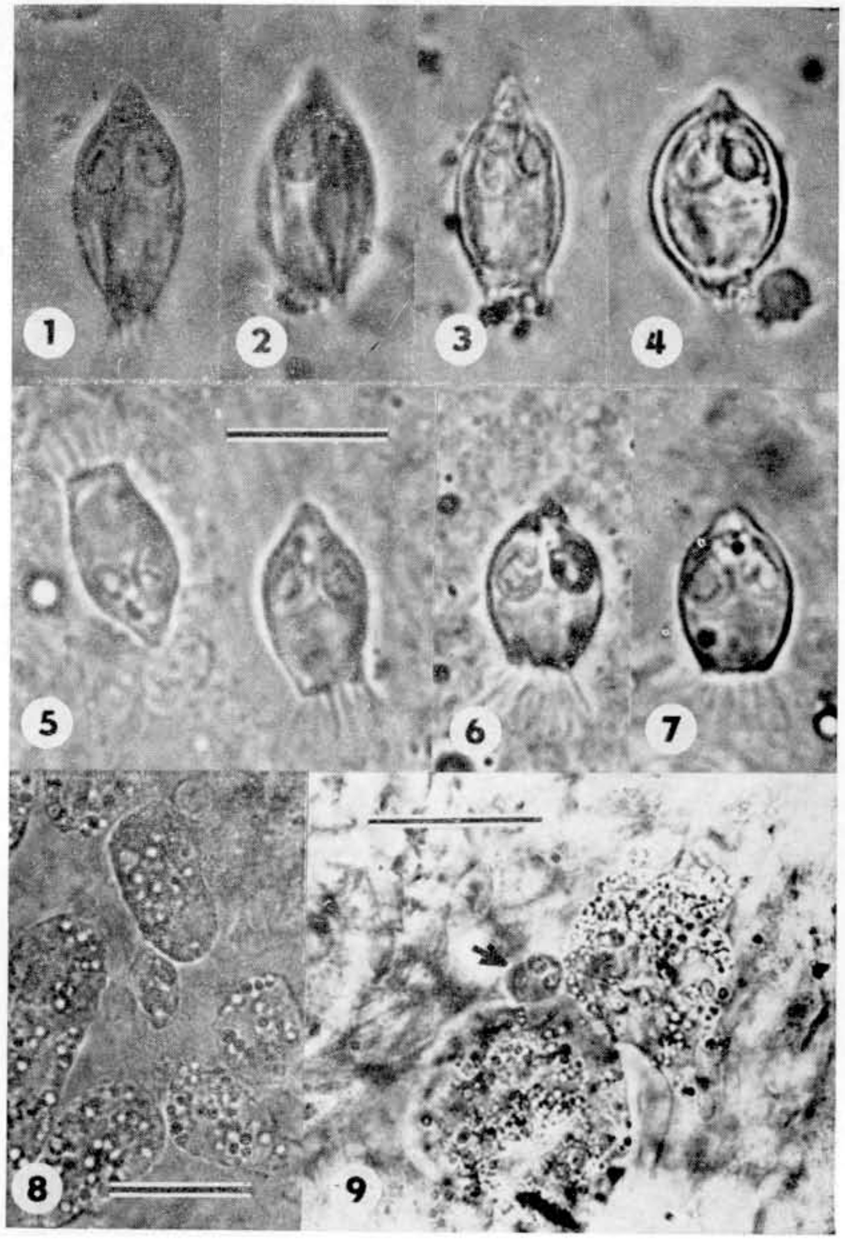
J. L., Parazitologický ústav
ČSAV, Branišovská 31, 370 05
České Budějovice, ČSSR



Figs. 1—4 — fresh spores of *Hoferellus carassii* from *Carassius auratus auratus*. 1,3 — typical spores; 2 — surface striation visible; 4 — anomalous, rounded spore. **Figs 5—7** — fresh spores of *H. carassii* from *C. auratus gibelio*. 5 — a typical spore; 6, 7 — more stubby ones. The bar in Fig. 5 equals 10 µm and applies to Figs. 1 through 7. **Fig. 8** — Small, growing plasmodia from *C. auratus gibelio*, one spore can be seen. Bar = 20 µm. **Fig. 9** — *H. cyprini* from carp. Two small plasmodia and one mature spore (arrow). Bar = 30 µm.

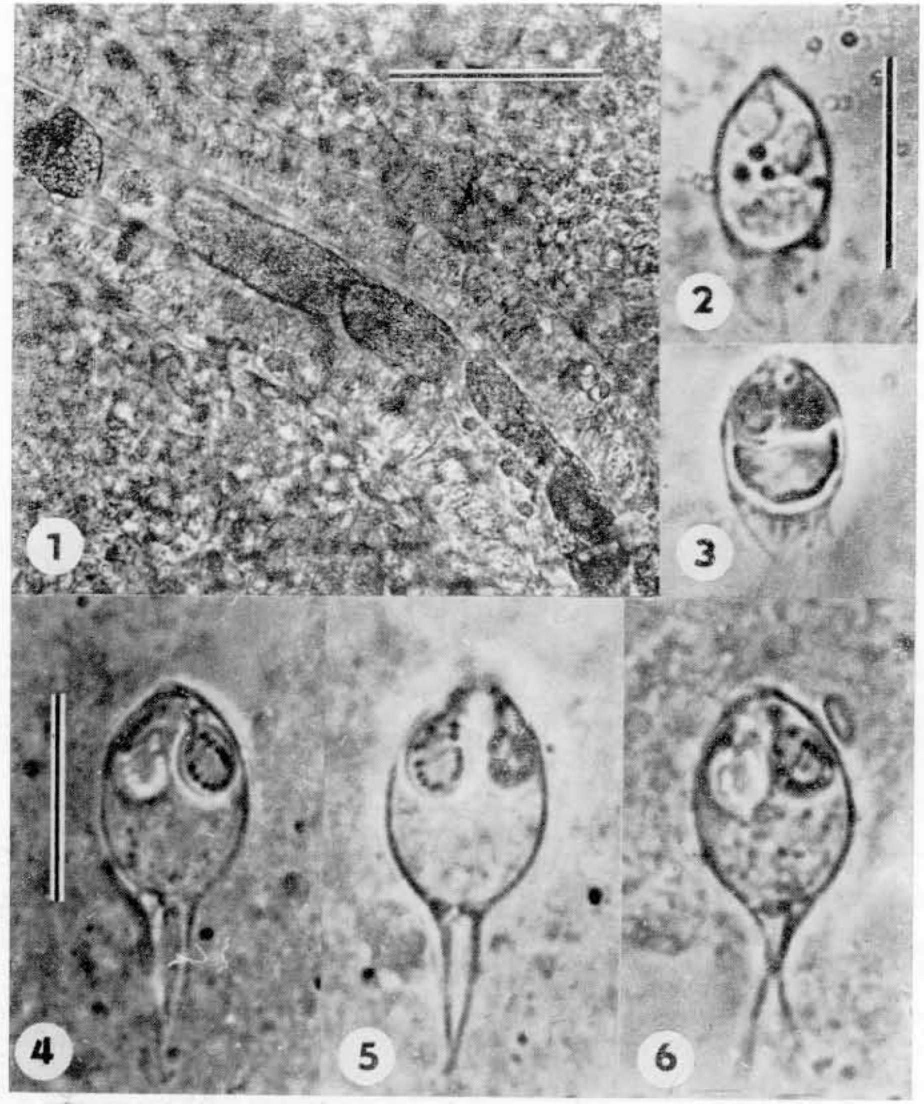


Fig. 1 — *Hoferellus cyprini*, elongated plasmodia without spores in the renal tubules of carp. **Bar** = 100 µm. **Figs. 2, 3** — two spores of *H. cyprini* from carp. **Fig. 2** shows typical outline of the spore in sutural view, the interior structure is damaged. **Fig. 3** is a rather anomalous spore with rounded posterior end. Bar = 10 µm. **Figs. 4—6** — fresh spores of *Myxobilatus nostalgicus*. Bar = = 10 µm.