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Myxosporeans infecting the gills of bigmouth buffalo (*Ictiobus bubalus*) in Illinois, USA

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Abstract. Four myxosporean species were found on the gills of *Ictiobus bubalus* from Illinois (USA). *Myxobolus endovasus* (Davis, 1947) Grinham et Cone, 1990 is revised. Three new species are recorded. *Myxobolus enoblei* sp. n. has spores ovoid in frontal view, 14.3 × 13 μm in size. *Myxobolus morrisonae* sp. n. has spores subcircular in frontal view, 10 × 9.5 μm in size; the surface of shell valves appears hairy when studied by SEM. *Triangula illinoisensis* sp. n. has spores rounded semicircular in frontal view, 10.2 × 12.8 μm in size. *Triangula illinoisensis* is the fourth species of its genus to be described from fishes.

Myxosporean parasites of North American freshwater fishes have been the subject of numerous studies (e.g., Gurley 1893, Ward 1919, Kudo 1919, 1929, 1934, Meglitsch 1937, Fantham et al. 1939, Li and Desser 1985, Grinham and Cone 1990) which have resulted in many species descriptions. It appears, however, that there are many more species than those known to date. In autumn of 1969 and in the spring of 1970, one of us (J.L.) had the opportunity to examine some Illinois freshwater fishes. This paper – with a very long delay – describes myxosporeans found in the bigmouth buffalo, *Ictiobus bubalus* (Rafinesque). Since this is a commercially interesting fish species, the knowledge of all its potential pathogens is quite desirable.

MATERIALS AND METHODS

Fish were collected by seining in a small creek near Allenville, Illinois and brought alive to the laboratory. All of their organs were examined macroscopically and tissue samples were inspected in fresh mounts under the microscope. Myxosporean spores were observed, measured (n = 25) and drawn fresh. The presence of mucous envelopes was examined with India ink (Lom and Dyková 1992). Fresh spores were photographed using the agar layer method (Lom 1975). For the scanning electron microscopy (SEM), spores were fixed in Parducz's fixative (Marszalek and Small 1969) for 6 min, freeze-dried, coated with gold and examined in the Cambridge Stereoscan Mark III electron microscope operated at 10 and 20 kV accelerating voltage.

RESULTS

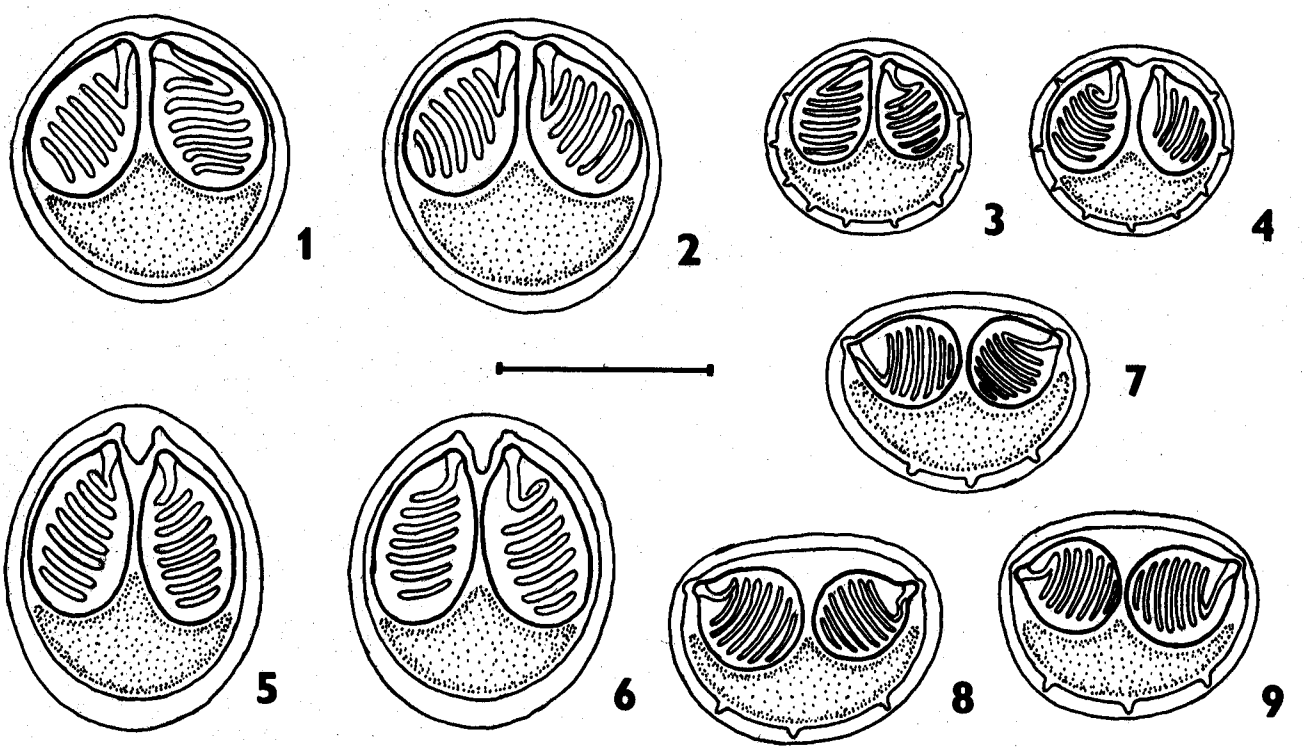
Myxobolus endovasus (Davis, 1947) Figs. 1–2, 10

Host: *Ictiobus bubalus*, bigmouth buffalo.
Site of infection: gill filaments.
Prevalence: 3 fish infected out of 11 examined.
Locality: Allenville, Illinois, USA; October 1969.

Plasmodia up to 1.2 × 1.3 mm in size were located in the gill epithelium mostly at the tip of the gill filament.

Spores were in front view broadly oval or almost spherical, length 14.3 (13.1–15.4) × width 13 (12.2–13.8) μm, thickness averaged 8.5 μm. Sutural edge not very prominent without any sutural markings. Ellipsoid polar capsules converged with their tapered anterior ends. Their size was 8.7 (7.7–9.6) × 5.5 (5.0–6.2) μm. There were 6 (sometimes 5 or 7) tightly coiled turns of the polar filament, situated at an angle of 30° (or less) to the long axis of the polar capsule. The capsule's hind end reached or exceeded the mid-spore length. The intercapsular appendix resembled a tiny tubercle. There was a weakly staining iodinophilous vacuole and no mucous envelope.

Remark: In the original description, Davis (1947) recorded smaller dimensions of the spore – 9 × 8 μm – and of polar capsules – 5 × 3.5 μm. His material was, however, fixed in formalin and the shrinkage – and possibly variability of populations – may account for the smaller size.



Figs. 1–9. Diagrammatic drawings of fresh spores in frontal view. **Figs. 1, 2.** *Myxobolus endovasus* (Davis, 1947). **Figs. 3, 4.** *Myxobolus morrisonae* sp. n. **Figs. 5, 6.** *Myxobolus enoblei* sp. n. **Figs. 7–9.** *Triangula illinoisensis* sp. n. Bar = 10 μm.

Myxobolus morrisonae sp. n. Figs. 3, 4, 11–12

Host: *Ictiobus bubalus,* bigmouth buffalo.
Site of infection: gill filaments.
Prevalence: 3 fish infected out of 5 examined.
Locality: Allenville, Illinois, USA, April 1970.

Elongated plasmodia were situated in the axis of the gill filament. They were up to 1.5 × 0.3 μm.

Spores were ellipsoidal in front view, their size was 10 (9.6–10.5) in length × 9.5 (9.1–10.3) μm in width. Thickness was 5 μm. The sutural edge sometimes had markings around the posterior and side margin. There was no intercapsular appendix, just a slightly thickened sutural edge in its place. Oval, anteriorly tapering polar capsules had converging anterior ends; they reached into the posterior part of the spore. Their size was 5.5 (5.3–5.8) × 3.7 (3.4–4.0) μm. Six tightly coiled turns of the polar filament were situated at an angle of 30° or less to the long axis of the spore. There was no mucous envelope or an iodinophilous vacuole.

With SEM, the surface of the spores looked hairy as if covered with short, tiny hair-like projections.

Taxonomic affinities. This species clearly differs by various spore characters from those recorded thus far from bigmouth buffalo, i.e., *Myxobolus bubalis* Otto et Jahn, 1943, *M. discrepans* Kudo, 1920, *M. endovasus* (Davis, 1947), *M. ovatus* Kudo, 1934 and *M. transovalis* Gurley, 1893.

It cannot be identified with any of the Illinois myxoboli described by Kudo (1934). By subcircular outlines in front view our species resembles *M. congesticus* Kudo, 1934 from *Moxostoma anisurum, M. squamosus* Kudo, 1934 from *Nocomis biguttatus* and *N. micropogon* and *M. obliquus* Kudo, 1934 from *Carpiodes velifer* in which species the polar capsules only reach mid-spore length, apart from other differences.

Fantham et al. (1939) described numerous *Myxobolus* species from Canadian freshwater fishes. However, the descriptions are not accurate enough to allow for a detailed comparison, e.g., *M. subcircularis* Fantham, Porter et Richardson, 1939 from *Catostomus commersoni.* Meglitsch (1937) established the species *Myxobolus rotundus* from the gills of *Carpiodes cyprinus* which was renamed as *M. meglitschi* Grinham et Cone, 1990. It has similar subcircular spores; however, they are larger and have no sutural markings.

To our knowledge, no other *Myxobolus* examined thus far with SEM possesses a hairy surface. Since the number of such data in *Myxobolus* is limited, this feature cannot be used for species differentiation.

We propose to establish this species as a new one, *Myxobolus morrisonae* sp. n., in honour of Dr. Carol Morrison, an eminent fish pathologist and protozoologist.

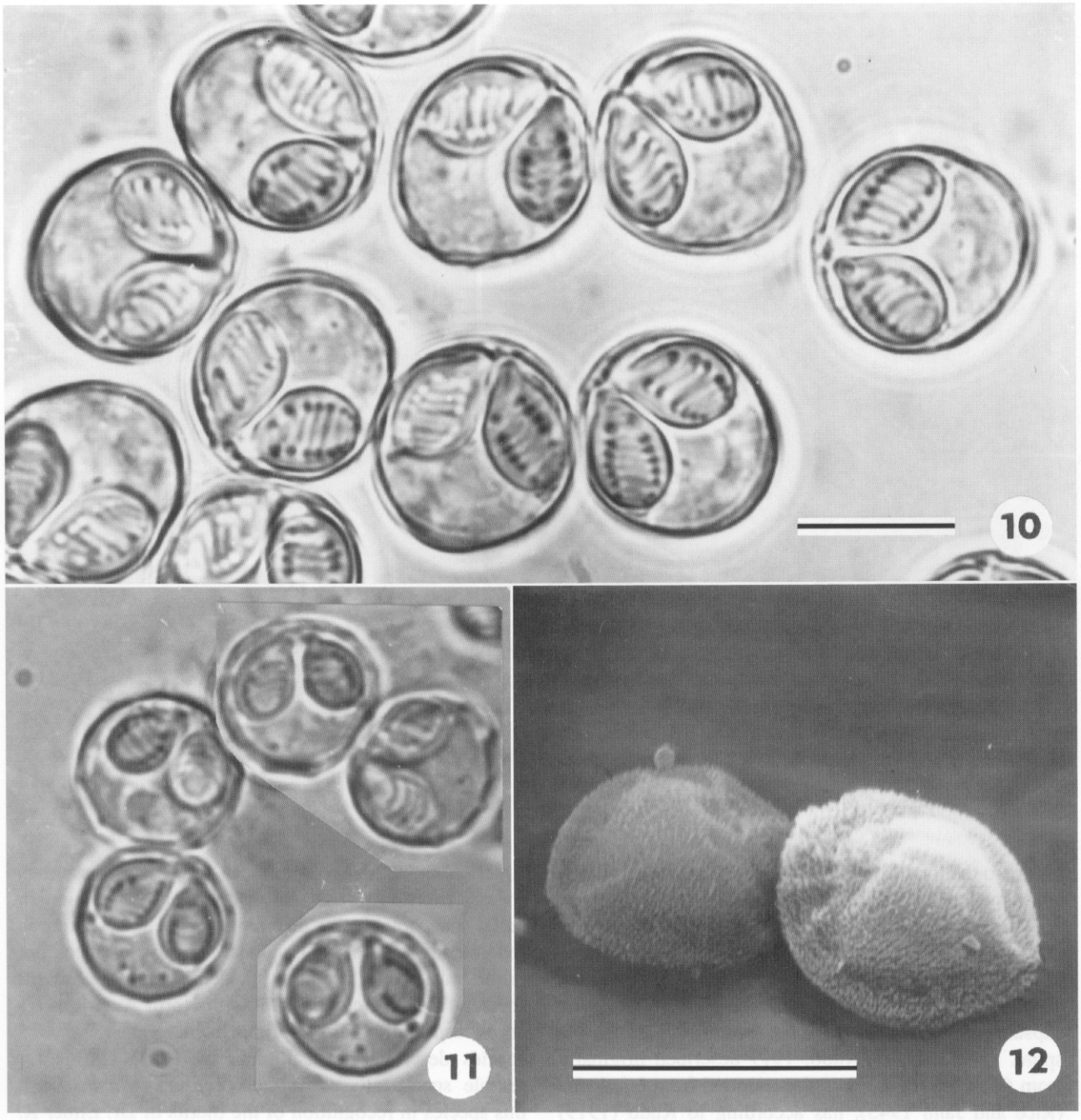


Fig. 10. Fresh spores of *Myxobolus endovasus* (Davis). Bar = 10 μm. **Fig. 11.** Fresh spores of *Myxobolus morrisonae* sp. n. magnified to the same scale as Fig. 10. **Fig. 12.** SEM micrograph of *M. morrisonae*, bar = 10 μm.

Myxobolus enoblei sp. n. Figs. 5, 6, 13

Host: *Ictiobus bubalus*, bigmouth buffalo.
Site of infection: gill filaments.
Prevalence: 4 infected out of 6 examined.
Locality: Allenville, Illinois, USA, April 1970.

Plasmodia were located within the axis of the gill filaments; they were elongated, up to 1.5 × 0.3 mm in size.

Spores were elliptical in front view, with an insignificantly narrower anterior end, length 14.4 (13.5–15.0) × width 11.1 (10.5–11.5) μm and 7.5 μm thick. A thick sutural edge bore delicate markings along the posterior end of the spore. Polar capsules were elongate oval and only slightly converged with their tapered anterior ends and reached deep into the posterior half of the spore. Intercapsular appendix was well formed. Polar capsule size was 8.3 (7.9–8.5) × 4.8 (4.5–5) μm. Polar filament was coiled in 7 turns (rarely 6 turns) at an angle of 30°

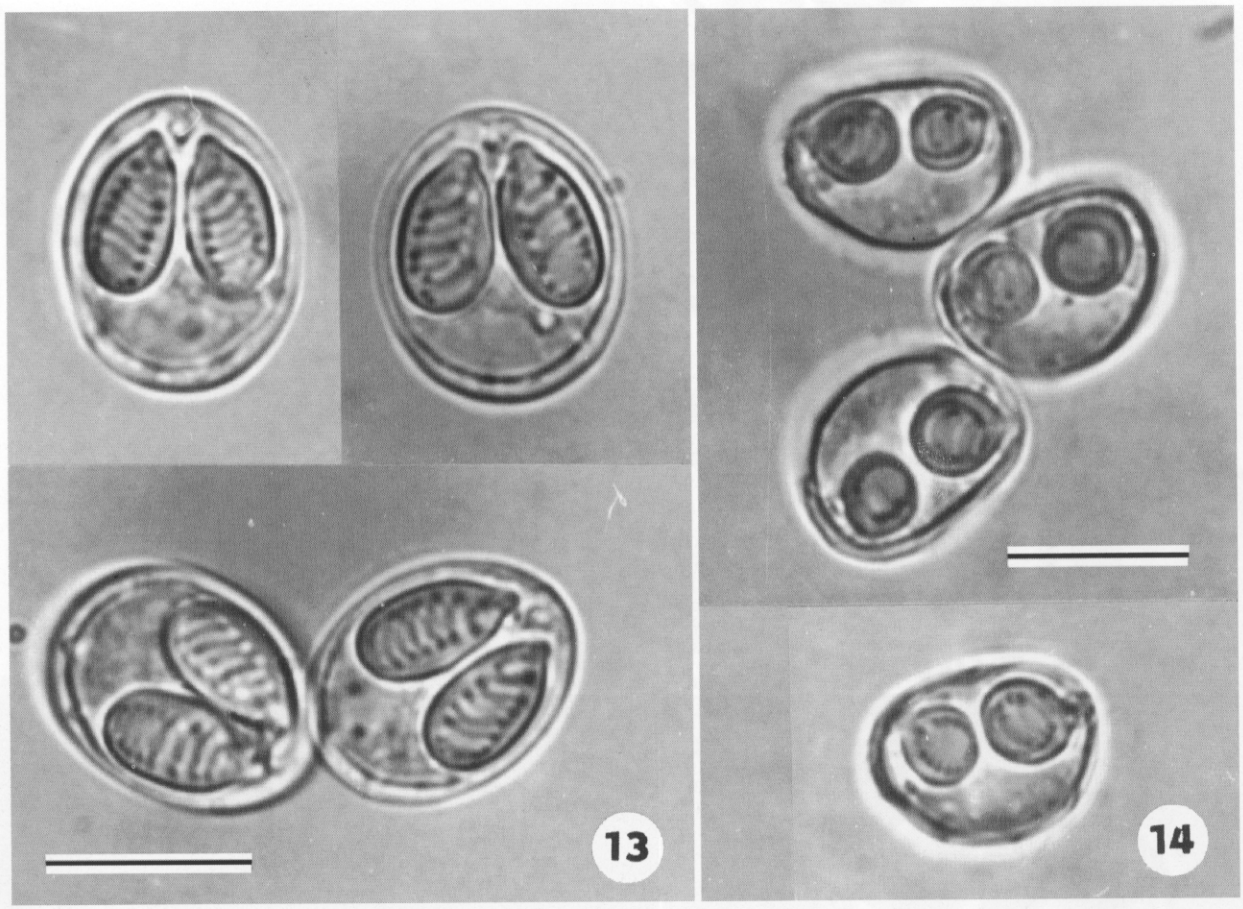


Fig. 13. Fresh spores of *Myxobolus enoblei* sp. n. **Fig. 14.** Fresh spores of *Triangula illinoisensis* sp. n. Bars = 10 µm.

or less to the long axis of the capsule. There was no mucous envelope or iodinophilous vacuole.

Taxonomic affinities. The present species clearly differs from others recorded in the same host. *M. bubalis* Otto et Jahn, 1943 was found to infect the intestine and has spores of a more elongated shape, with polar capsules not reaching into the posterior part of the spore. *M. discrepans* Kudo, 1919 described originally from *Carpiodes velifer* but also reported from *I. bubalus* (Rice and Jahn 1943), has according to Kudo (1919) about the same spore dimensions (although he measured fixed spores). However, the spore is attenuated posteriorly and the polar capsules are less elongated and barely reach mid-spore length. *M. ovatus* Kudo, 1933 infecting the skin has slightly smaller spores with thickenings on the posterior spore margin, without any intercapsular appendix and with narrower polar capsules. *M. transovalis* Gurley, 1893 described originally from *Clinostomus funduloides* was later also found in *I. bubalus* (Rice and Jahn 1943) clearly differs by spores that are wider (8 µm) than long (6–7 µm).

The spore of *M. enoblei* has no striking features and resembles many *Myxobolus* species from North America and other geographical areas. The numerous species from outside of North America, in addition to being morphologically different, can also be taken as separated by natural geographical barriers and need not to be taken into account in identifying the present species. We have compared it with North American records, especially from cyprinid hosts. None of the 16 species described from Illinois by Kudo (1934) resemble specifically *Myxobolus enoblei.* We establish this species in recognition of the merits of Dr. Elmer R. Noble in the research of myxosporean parasites.

Triangula illinoisensis sp. n. Figs. 7–9, 14

Host: *Ictiobus bubalus*, bigmouth buffalo.
Site of infection: gill filaments.
Prevalence: 2 fish infected out of 5 examined.
Locality: Allenville, Illinois, USA, October 1969.

Elongated plasmodia up to 1.4 × 0.2 mm were localised in the secondary gill filaments.

Spores had in front view an asymmetrical oval shape, the anterior margin was almost flat while the posterior margin was considerably vaulted or appeared as if broken or uneven or it had up to 3 sutural markings. Almost subspherical polar capsules, 4.9 (4.4–5.2) × 4.2 (3.9–4.5) μm with tapered apices discharging in opposite directions at the end of the spores. There were 6 to 8 tightly joint turns of the polar filament at an angle more or less perpendicular to the long axis of the capsule. There was no mucous envelope or an iodinophilous vacuole.

Taxonomic affinities: Polar capsules, set widely apart, and other features of the spore suggest that this species is a member of one of the three further mentioned genera. *Neomyxobolus* Chen et Hsieh, 1960, *Ortholinea* Shulman 1962, and *Triangula* Chen et Hsieh, 1984 cannot be clearly separated by the known characters of the various species. However, unless their mutual relations are duly revised, we prefer to continue to recognize them as separate taxa. The two species of the genus *Neomyxobolus* are known from the urinary tract of freshwater fishes and have spores with ridged surface. Although the spores have a comparable shape, the histozoic character of the present species is at variance with the habitat of *Neomyxobolus*.

Some species of *Ortholinea* have spores with a smooth surface; however, all are coelozoic in marine fishes and therefore it is reasonable not to consider this genus for the present species. *Triangula* with its histozoic species forming spores with a smooth surface and inhabiting freshwater fishes is a better choice. *T. percae* Langdon, 1987 differs according to the original description (Langdon 1987) both in spore shape and site in the host. The two remaining species both infect gills. *T. yangkiangensis* Chen et Hsieh, 1984 differs in spore shape, host and geographic distribution (China – Chen and Hsieh 1984). *T. percotti* (Akhmerov, 1960) originally described as a *Sphaerospora* by Akhmerov (1960), has similar spores but also differs in host and locality in East Asia. Therefore we propose the present species as *Triangula illinoisensis* sp. n. The specific name is derived from the state where it was found.

CONCLUDING REMARKS

Landsberg and Lom (1991) compiled a list of the then known 444 species of the genus *Myxobolus*. Taking into account several unfortunate facts – the enormous number of species (diagnosed according to mostly very similar spores), very often insufficient or sloppy descriptions, unknown host or tissue specificity – one has to accept a certain degree of uncertainty of the many established species. In addition, the geographic range of many species is also not well known; e.g., the Eurasian species *Myxobolus muelleri* Bütschli, 1882 has been reported to exist in North America (Mitchell 1989) and this may be true for other species. Also, there is a great variability of species, a persuasive example of which is the same *M. muelleri* (Davies 1968, Mitchell 1989). At the same time, this variability cannot be verified experimentally since experimental infections via the tubificid worms are not easy (see review in Kent et al. 1994). In spite of all these reservations, it is necessary to proceed with classification of myxosporea to reach better understanding of parasite fauna of fish.

In some instances, differentiation of species may be facilitated by the use of scanning electron microscopy. While it is indispensable in the genus *Chloromyxum* (Lom and Dyková 1993) surface patterns of *Myxobolus* spores are rather limited. Their valves are mostly smooth or artificially wrinkled due to fixation; there may be just a few perisutural ridges or grooves (e.g., Lom and Hoffman 1971, Amandi et al. 1985, El-Matbouli et al. 1990). Thus exceptional features like hairy surface in *M. morrisonae* may be of great help.

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