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Myxidium leei n. sp., a pathogenic myxosporean of cultured sea bream *Sparus aurata*

Ariel Diamant[1], Jiří Lom[2], Iva Dyková[2]

[1] Israel Oceanographic & Limnological Research Ltd., National Center for Mariculture, PO Box 1212, Elat 88112, Israel
[2] Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

ABSTRACT: *Myxidium leei* n. sp. is described from cultured sea bream *Sparus aurata* L. in Cyprus and Israel. It is histozoic in the intestinal wall and forms small plasmodia that give rise to 2 spores each. The average size of fixed spores is 14.7 µm in length and 6.9 µm in width; their shape is arcuate, elongated polar capsules (average size 3.2 × 7.4 µm, with 7 turns of the polar filament) open at one side of the spore. The case of this species, which reveals a combination of taxonomic characters fitting both *Myxidium* and *Zschokkella*, stresses once again the non-existence of a sharp morphological boundary between the 2 genera.

KEY WORDS: *Myxidium leei* · Myxosporea · Taxonomy · Pathology · *Sparus aurata* · Mariculture

INTRODUCTION

In 1992, Diamant reported a new pathogenic myxosporean which caused an outbreak of mortalities in gilt-head sea bream *Sparus aurata* L. cultured at a fish farm in southern Cyprus. Recently, the parasite was also found in cultured *S. aurata* in Israel (Diamant unpubl. data). The species was identified as belonging to the genus *Myxidium*. It infested the intestinal mucosa, which was densely pervaded by developmental stages. The present paper deals with the taxonomic position of the parasite. This is of significance, because of the parasites's pathogenic potential and histozoic way of life. Only a few of the known species of the genus *Myxidium* are known to be histozoic.

MATERIALS AND METHODS

Tissue samples were obtained from pond-reared *Sparus aurata* in Cyprus (1992) and Israel (1994). Spores fixed in buffered neutral formalin (BNF) were studied, measured and photographed. The viscera of the affected fish were fixed in 10% BNF and processed for paraffin histology. Sections were stained with hematoxylin-eosin.

For electron microscopy, infected tissue was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and embedded in Epon 812 or Epon-Araldite resins. Ultrathin sections were double stained and details of the spore structure were observed in a Jeol JEM 100CXII transmission electron microscope.

RESULTS

Myxidium leei n. sp.

The parasite develops in the form of small plasmodia; in sectioned material, their average size was 22 µm. The plasmodia, which are disporic, are wedged between the epithelial cells of the mucosa along the entire length of the intestine, from the pylorus to the rectum (Fig. 1). Most of them are located between the basal parts of the epithelial cells, while others are situated at the level of the nuclei of these cells. The resulting pathology was as previously described (Diamant 1992). Close examination of various organs from 5 affected fish (liver, gall bladder, spleen, kidney, urinary bladder, heart, stomach and gills) revealed some spores of the parasite in the urinary bladder of one of the fish.

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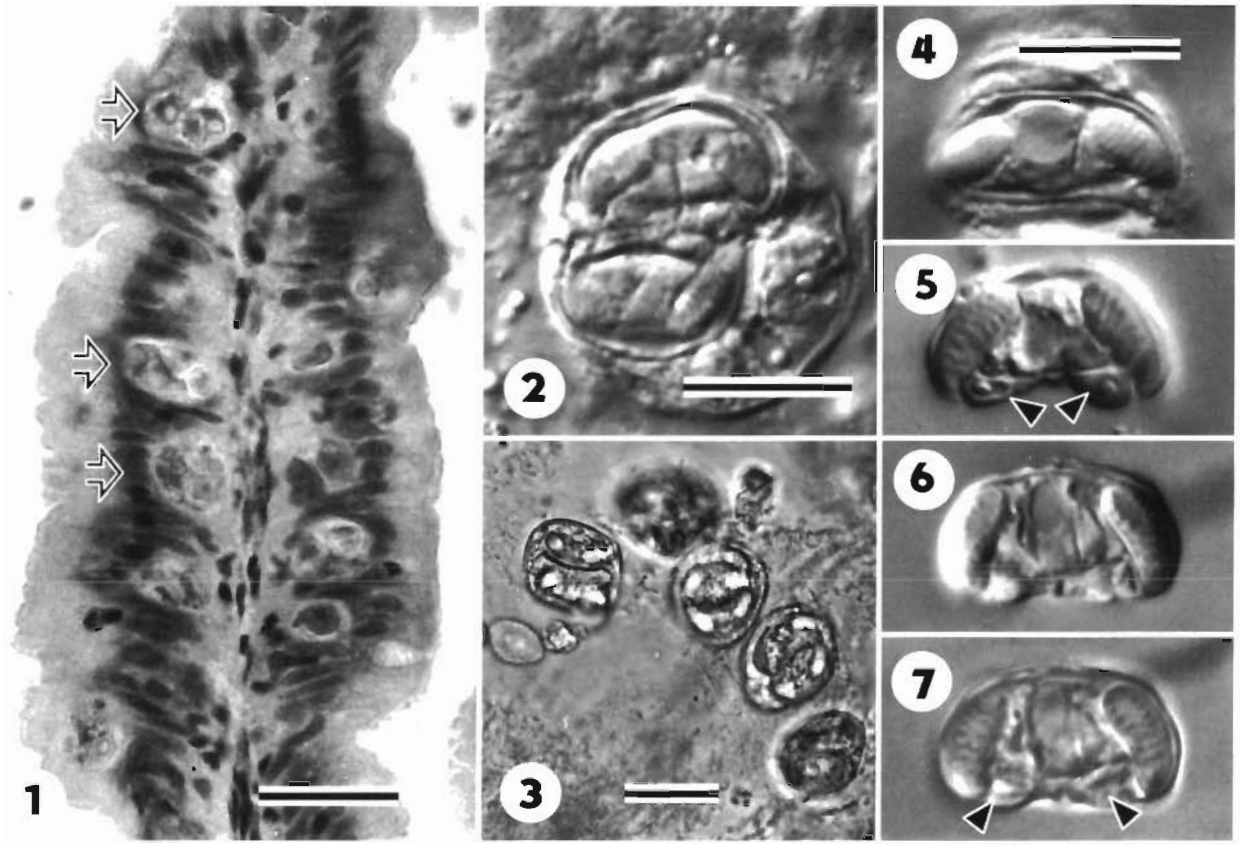

Figs. 1 to 7. *Myxidium leei* n. sp. infecting *Sparus aurata*. Fig. 1. Section of an infected intestinal villus with developing parasite stages (arrows). H&E, scale bar = 20 µm. Fig. 2. Pair of immature spores within the plasmodium. 10% neutral formalin fixed wet mount, scale bar = 10 µm. Fig. 3. Several plasmodia with developing spores. Neutral formalin fixed wet mount, scale bar = 20 µm. Figs. 4 to 7. Spores; scale bar = 10 µm. Fig. 4. An immature spore still within plasmodial remains. Figs. 5 to 7. Mature spores, arrowheads point at the bulging remnants of the valvogenic cells. Figs. 4 to 6: Nomarski interference microscopy; Fig. 7: phase contrast microscopy

The spores (Figs. 2 to 8) have an arcuate, almost semicircular shape, the size of formalin-fixed spores was 6.9 (5.6 to 7.8) µm in width (n = 15) and 14.7 (13.2 to 15.2) µm in length; average thickness was 6 µm. The surface of the spores reveals, on close inspection, an extremely fine longitudinal striation. Electron microscopy reveals that during morphogenesis, a large mass of lipid-like vacuoles is present in the capsulogenic cell (Fig. 8). At the side of the spore, which appears concave in frontal (valvular) view, close to the polar capsules are 2 bulges, remnants of the valvogenic cells. They diminish in size as the spore approaches maturity. Elongated polar capsules, tapering to their distal ends, open at one side of the spore, diverging at an angle of about 90°, and are 7.4 (6.2 to 8.8) × 3.2 (2.8 to 3.8) µm in size. There are, as a rule, 7 turns (range 6 to 8) of the polar filament coil. The distal end of each capsule is housed in a special projection of one of the 2 shell valves, since the suture line does not run regularly in the longitudinal midline around the spore length, but crosses the distal part of each capsule (Fig. 9). The suture is rather delicate and thus quite difficult to discern in fixed material; it is wavy or sinuous rather than straight and its course may be irregular. In frontal view, it runs above one of the capsules and underneath the other. The sporoplasm has the shape of an asymmetrical hourglass in front view; there is a large vacuole in its center.

DISCUSSION

There are 2 genera to which this species can be assigned: *Myxidium* Buetschli, 1882 and *Zschokkella* Auerbach, 1910, but in either case with some reservations. According to the generic definition of *Myxidium* as understood today (see Lom & Noble 1984), the genus is characterized, as a rule, by fusiform, straight, slightly crescent or sigmoid spores with more or less pointed ends, and with a straight or sigmoid sutural line bisecting the spore. The polar capsules are pyriform to tear-like, open usually in opposite directions

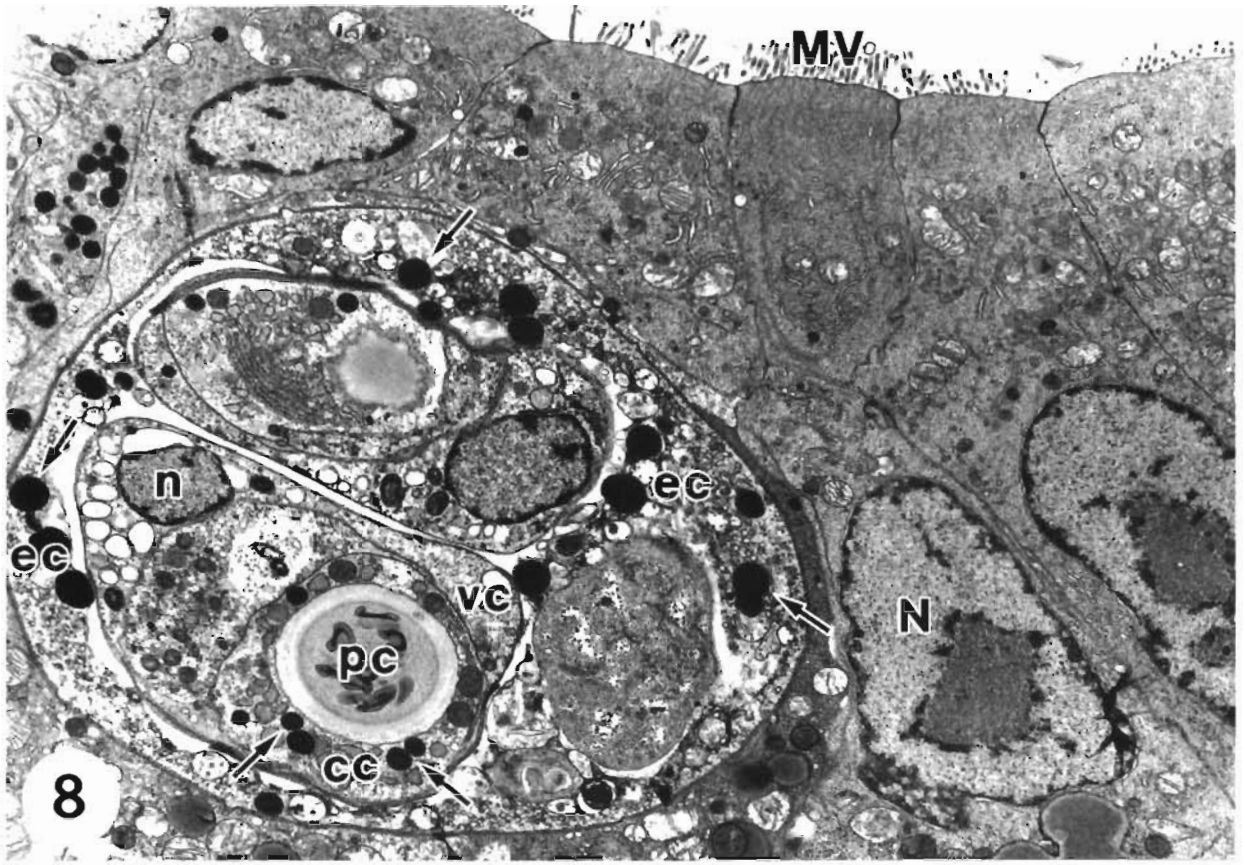

Fig. 8. *Myxidium leei* n. sp. infecting *Sparus aurata*. Electron micrograph of a *M. leei* plasmodium located in the gut epithelium of *S. aurata*. The pansporoblast consists of 2 developing spore units encompassed by an envelope cell (ec). In the spore at lower left, a developing polar capsule (pc) lies within its capsulogenic cell (cc). Note electron-dense vacuoles located within the capsulogenic cell as well as envelope cell cytoplasms (arrows). A small portion of nucleus (n) is visible in a valvogenic cell (vc) that surrounds the capsulogenic cell. At right, the nucleus (N) as well as surface microvilli (MV) of host epithelial cells are shown (×5900)

and their foramina lie terminally, very close to the sutural plane. In *Zschokkella*, spores are defined as mostly ellipsoidal in sutural view, often slightly bent in frontal view, with rounded or bluntly pointed ends. The sutural line is straight to sinuous. Polar capsules, mostly near-spherical, open subterminally and both to one side.

The present species does not, therefore, correctly fit the definition of either of the 2 genera. On one hand, the polar capsules are located terminally and are elongated rather than subspherical (as in *Myxidium*), but on the other hand, they open to one side and their foramina are not located in the sutural plane (as in *Zschokkella*); the sutural line does not bisect the spore but is quite wavy, and does not reach the spore extremities (as in many *Zschokkella*). The case of our species falls squarely in line with the known fact that the distinction between *Myxidium* and *Zschokkella* is rather precarious and some species are difficult to assign to either of the two. There are many more examples to illustrate this. *M. triangulum* has rounded triangular or almost semicircular spores in frontal view, and bears very little resemblance with the type species *M. lieberkuehni* Buetschli, 1882 which has slender fusiform spores, while its shape is quite close to the

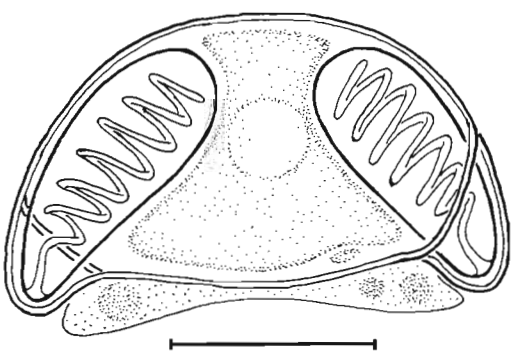

Fig. 9. *Myxidium leei* n. sp. infecting *Sparus aurata*. Spore in frontal view; scale bar = 5 µm

type species of the genus *Zschokkella*, i.e. *Z. hildae* Auerbach, 1910. On the other hand many *Zschokkella* species, e.g. *Z. nova*, have oval spores quite dissimilar to *Z. hildae*. In view of the not unambiguous character of our findings and of the rather hazy distinction between *Myxidium* and *Zschokkella*, a due differential diagnosis is required for comparison of the present species with both *Myxidium* and *Zschokkella*.

In *Myxidium*, the known few histozoic species that occur in freshwater hosts do not endure estuarine environments and have no known contact with marine fish; in addition, these species are morphologically different. Different spores occur also in *M. giardi* Cépède, 1906 (see Lom & Dyková 1992) and *M. matsui* Fujita, 1929 (see Fujita 1929), histozoic parasites of eels *Anguilla anguilla*, *A. japonica*, *A. dieffenbachi*, *A. rostrata* (*M. giardi*) and *A. japonica* (*M. matsui*), where some contact with marine fish is conceivable. Since there is a possibility that some Myxidia may be in their hosts as both coelozoic and histozoic forms (e.g. *M. giardi*, Lom & Dyková 1992; *M. rhodei*, Dyková et al. 1987) we cannot completely dismiss the possibility that some of the already recorded marine *Myxidium* species may be identical with the present species. However, as evident from the review by Jayasri & Hoffman (1982) and from an exhaustive search of the literature, species with spores of about the same dimensions have a different spore shape.

In the genus *Zschokkella*, to the best of our knowledge, there are 4 histozoic species. Three were described by Chen & Hsieh (1984) from Chinese freshwater fishes and are morphologically different. A fourth species, described by Fantham et al. (1939) as *Z. salvelini* from the kidney tissue of *Salvelinus fontinalis*, has spores similar to, but not identical with, our finding. None of the coelozoic *Zschokkella* species found in marine fishes is identical to ours either.

We have mentioned that the present parasite, found in *Sparus aurata*, has spores with features unequally divided between those of *Zschokkella* and those of *Myxidium*. Generic differences between spores of *Myxidium* and *Zschokkella* appear satisfactory as long as we do not try to verify to what extent the species assigned to these genera comply with the generic characterization as stated above. Then we can find that many differ from the type species or defy the definition altogether For example, 'spherical' polar capsules is a rather ambiguous feature. Capsules that are genuinely near-spherical, with only a tiny pointed apex, can be found, for example, in the genus *Ceratomyxa*. In the type species *Z. hildae* in Auerbach's (1910) original drawing the capsule seen from the side appears to be drop-shaped, i.e. tapering to a markedly protruding point at one end: similar drop-shaped capsules are seen in the description of *Z. embiotocidis* by Moser & Haldorson (1976). Polar capsules which appear more spherical, i.e. their tapered apex is less prominent, have been demonstrated, e.g. in *Z. orientalis* Konovalov and Shulman, 1966 or *Z. nova* and in most other *Zschokkella* species. In addition, such polar capsules were also described in *Myxidium* species, e.g. in *M. macrocapsulare* Auerbach, 1910, *M. truttae* Léger, 1930, *M. ventricosum* Shulman, 1962, and *M. triangulum* Shulman, 1962, and spherical capsules were recorded in *M. schulmani* Chernova, 1970; Chen (1973) described them in *M. spinosum* Lee & Nie, 1965; Chen & Hsieh (1984) recorded such spherical capsules when establishing *M. neimongoli* and *M. ochengensis*. The confusion between *Zschokkella* and *Myxidium* was also the reason why *Zschokkella ophiocephali* Chen & Hsieh, 1961 with almost spherical polar capsules was later shifted to the genus *Myxidium* (see Chen 1973).

Similar inconsistency also exists in the direction in which the polar capsules discharge their filaments; unlike *Zschokkella hildae*, many *Zschokkella* species discharge their filaments in diagonally opposite directions (e.g. *Z. costata* Kashkowsky, 1965 or *Z. mugilis* Sitjá-Bobadilla & Alvarez-Pellitero, 1993) or in an opposite or axial direction (e.g. *Z. orientalis* Konovalova & Shulman, 1966 or *Z. parasiluri* Fujita, 1927). On the other hand, all *Myxidium* species that have crescent-shaped spores discharge their filaments to one side, e.g. *M. kagayamai* Kudo, 1919 or *M. melanostomi* Naidenova, 1970. *M. triangulum* Shulman, 1962 and *M. monstruosum* Shulman, 1962 not only have bulky spores that resemble *Z. hildae* in frontal (valvular) view, but also discharge their filaments more or less to one side. All this shows, as stated previously (Lom & Noble 1984), that the distinction between *Myxidium* and *Zschokkella* is quite artificial and boundaries between the genera are arbitrary. Because of pronounced tear-like shape of the polar capsule, as yet not shown in *Zschokkella*, and since the genus *Myxidium* was established earlier than *Zschokkella* (i.e. in the case of a potential fusion of the 2 genera it is *Myxidium* which is to be preserved), we assign the *Sparus aurata* parasite to the genus *Myxidium*. We propose to establish it as a new species, *M. leei* n. sp., in honor of Professor John J. Lee of the City College of New York, NY, USA. In our opinion, it would be premature now to lump the 2 genera together, and it would be advisable to wait for the results of transmission experiments to see what differences, if any, exist between the 2 genera. Thus far, Benajiba & Marquez (1993) have shown that *M. giardi* transforms into an actinosporean of the genus *Neoactinomyxon* in the only transmission experiments performed with species of either of these 2 genera.

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