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Microsporidia sp. in Atlantic bluefin tuna (*Thunnus thynnus*)

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Abstract

During a parasitological examination of Adriatic cage-reared young tuna, small and whitish xenomas were observed and isolated from the intestinal mucosa. Because of their strong resemblance to didymozoid cysts, xenomas were ruptured and fresh and stained smears were prepared. However, the microsporidian species was not determined in this study. This is the first record of a microsporidian parasite in tuna. Given the recognised potential pathogenicity of this parasitic group in wild and cultured fish populations, and the high value of reared tuna, it is important to continue with further research.

Introduction

Microsporidia are among the most taxonomically controversial obligate intracellular parasitic eukaryotes. Even though these rather simple-structured parasites were recognized as pathogens in the nineteenth-century, being reported as parasites of silkworms and afterwards other invertebrate and vertebrate hosts, it is only recently that they have been isolated as the causative agent of diseases in humans (Desportes et al., 1985). Once thought to belong among the earliest branches on the eukaryotic tree, recent molecular methods have revealed their origin as fungal (Fischer & Palmer, 2005).

Microsporidian ultrastructure was considered to be the most reliable species discrimination feature, however, molecular studies suggest that it does not reliably determine the phylogenetic relationships between genera (Lom & Nilsen, 2003), and that the habitat and host type are the main mechanisms that

determine the placement of species in groups or clades in the Microsporidia (Vossbrinck & Debrunner-Vossbrinck, 2005). The best example was given with *Ichthyosporidium* and *Loma salmonae*; structurally very different even though in the phylogenetic sense they group together (Nilsen, 2000).

Fish are hosts to approximately 156 Microsporidia species belonging to 14 genera (Lom & Nilsen, 2003). Microsporidia appear to be less host specific in fish hosts, even in those species that form xenomas (Lom & Dyková, 2005).

Pathological effects by Microsporidia on host tissues have been reported from wild and cultured fish (Kent, 2000; Yokoyama et al., 2002; Canning & Curry, 2005). The main feature is the hypertrophic growth of infected cells forming a xenoma, which are able to reach up to 14 mm in diameter. If located in musculature, xenomas cause liquefaction and decrease product quality. In Japan, serious

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damage was caused by microsporidial infection in the farmed yellowtail (*Seriola quinqueradiata*) and red seabream (*Pagrus major*), where infected musculature had a characteristic concave surface (Sano et al., 1998).

Fattening of bluefin tuna (*Thunnus thynnus*) was developed in Mediterranean in early nineties with the strong trend of geographic spread and growth of the production that has increased almost 15 fold during the past decade (Miyake et al., 2003). There are few reports of outbreaks of bacterial and viral diseases in cage-reared tuna (Mladineo et al., 2006) although there are abundant data on parasitic organisms in wild and reared tuna (Williams & Bunkley-Williams, 1996; Munday et al., 2003; Mladineo & Tudor, 2004).

During a parasitological survey of captured young tuna in the Adriatic Sea, unidentified microsporidial xenomas were isolated from the intestinal mucosa, making this case a first report of the microsporidian in tuna.

Materials and methods

Fish weighing 6 ± 1 kg were caught in waters of the Island of Jabuka, trawled to the farm, kept in cages for three weeks, and fed on a diet of mixed fish from small trawling boats. Parasitological examination was done on daily mortalities of non-adapted fish. Samples of viscera were individually collected in plastic bags and transported to the laboratory, where fresh smears of gills, kidney, spleen, liver, gall bladder, intestinal and stomach mucosa and ventricular endocard were examined under a light microscope. Xenomas were measured, ruptured and observed under immersion and stained by Giemsa.

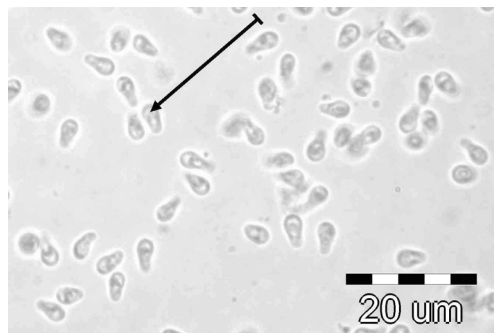


Figure 1. Fresh smear of microsporidian macrospore isolated from the intestinal xenoma in young Atlantic bluefin tuna (*Thunnus thynnus*). Arrow shows posterior vacuole.

Results

Microsporidian xenomas were isolated from the mucosa of the intestine, as whitish elliptical cysts, measuring 2.1×0.8 mm, resembling *Koellikerioides intestinalis* (Didymozoidae, Digenea) cysts, except in colour. One xenoma was isolated in each of two fish (N=6).

Ruptured xenomas showed the presence of mature macrospores, without sporophorous vacuole which may have resulted from the higher pressure employed in the rupture of the xenoma. Spores were elliptical to pyriform, measuring $2.46 \pm 0.28 \times 4.88 \pm 0.31$ μ m. In fresh smears, a posterior vacuole was observed as a translucent part occupying one third of the spore (Figure 1). In Giemsa smears, only centrally located sporoplasm with one nucleus was stained and observed (Figure 2). Three folds smaller microspores of the same shape were isolated from the second xenoma.

Using a practical key for the determination (Lom & Dyková, 1992), based on only light microscopy it could be speculated that this species belongs to *Loma* or *Glugea* sp.

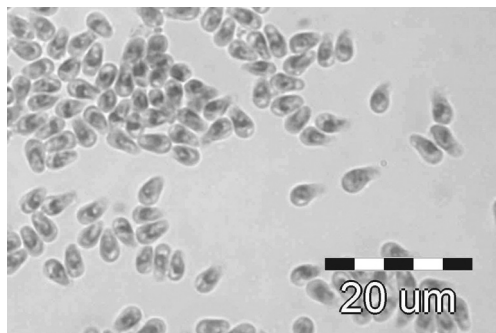


Figure 2. Giemsa stained smear of microsporidial macrospore isolated from the intestinal xenoma in young Atlantic bluefin tuna (*Thunnus thynnus*).

Discussion

This is the first report of a Microsporidian infection in tuna. Due to the lack of ultrastructural data, we have been unable to unequivocally place the parasite in a particular genus, although based on several features; it most probably belongs to either *Loma* or *Glugea*. In the genus *Loma*, *L. branchialis* infects mostly gills and pseudobranchs of gadoid fish, where the prevalence is considerably high, reaching up to 60% (Morrison & Sprague, 1981). Xenomas are also found in various visceral organs or connective tissue of the digestive tract, and are composed of spores and developmental stages intermingled throughout the xenoma. The genus *Glugea* comprises some 31 species and many of them form xenoma in the layers of the digestive tract of marine fish. The characteristics of the genus are multinucleate meronts, sporogonial plasmodia developing in the sporophorous vacuole, uninucleate sporoblasts and xenomas of up to 14 mm with branched nuclei at the periphery (Lom & Dyková, 1992). *Glugea machari* has been isolated from Adriatic common dentex

(*Dentex dentex*), which is similar in spore shape and in the measurements of the xenoma (after Jirovec, 1934), however the case was reported only once and it is more probable that tuna were infected during their migration across the Atlantic and Mediterranean Seas.

In the absence of ultrastructural morphology of the xenoma and of the spores, along with the description of meronts and sporoblasts, it is impossible to assign the isolated Microsporidia to a particular genus with certainty. Nevertheless, this is a first report of the microsporidian parasite in tuna and considering the commercial importance of reared tuna and the potential pathogenicity of this parasite further research is important and worthwhile.

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