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FIRST REPORT ON THE OCCURRENCE OF AN ACTINOSPOREAN STAGE (MYXOZOA) IN OLIGOCHAETES FROM SPANISH FRESHWATERS

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Oligochaetes living in the Mijares River close to the Sitjar reservoir, and polychaetes from the brackish and marine waters of a channel flowing into the Mediterranean Sea, both in the province of Castellón (Spain), were examined for the presence of actinosporea. An aurantiactinomyxon was isolated from 60 specimens of the oligochaete *Branchiura sowerbyi* collected from the river, but no actinospores were isolated from 160 polychaetes collected from the sea channel. The aurantiactinospores were detected by the cell-well plate method. The detected species are not identical with any of the aurantiactinomyxon forms hitherto described in the literature. This is the first report on the occurrence of an actinosporea in Spanish waters.

Key words: Actinosporea, Myxozoa, aurantiactinospore, *Branchiura sowerbyi,* Oligochaete, first report

The first description of Actinosporea was published a century ago by Stolc (1899), who described these organisms as parasites related to Myxosporea. However, only few researchers studied them during the subsequent years. Among these, Ikeda (1912) and Mackinnon and Adam (1924) detected tetractinomyxon and triactinomyxon forms. Janiszewska (1955, 1957) performed detailed studies on the morphology, ecology and systematics of Actinosporea. Research on Actinosporea gathered momentum after Wolf and Markiw (1984) demonstrated that the fish-parasitic myxosporean *Myxobolus cerebralis* had an actinosporean stage developing in *Tubifex tubifex.* Since then, there has been an avalanche of reports on actinosporean infections and studies on the life cycle of Myxosporea involving polychaetes (Bartholomew et al., 1997), and mainly oligochaetes from natural freshwaters and fish farms (Marques, 1984; Hamilton and Canning, 1987; Székely, 1989; Burtle et al., 1991; Styer et al., 1992; Pote and Waterstrat, 1993; Koller, 1994; Pallós, 1995; McGeorge et al., 1997; El-Mansy et al., 1998*a, b*;

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Xiao and Desser, 1998*a, b, c*). Works on actinosporean infections in marine waters are, however, scarcer (Roubal et al., 1997, Hallet et al., 1998, 1999). Thus, the objective of the present survey was to detect actinosporean stages in two different Spanish aquatic habitats.

Materials and methods

Samples were collected from the mud and the roots of the aquatic vegetation of two different habitats in the Province of Castellón (Spain), in the second half of June 1999. In the first one (A), oligochaetes were taken from the Mijares River, close to the Sitjar reservoir. In the second one (B), polychaetes were collected from a channel flowing into the Mediterranean Sea close to the Instituto de Acuicultura de Torre de la Sal (IATS). Water salinity in site B is very variable depending on the season and the rainfalls, and it ranged from 17 to 33‰ when samples were taken. The water reservoir was inhabited mainly by cyprinids, whereas mullets and European eels were common in site B.

Oligochaetes and polychaetes were examined for the presence of actinosporea by the cell-well plate method of Yokoyama et al. (1991). Briefly, worms were individually placed into plastic welled plates containing tap water or filtered seawater (34‰ salinity), respectively, incubated at 15 °C, and examined daily under an inverted microscope for the presence of actinospores released from the worms to the water. Seawater was changed in the wells every 1–3 days as needed (while the polychaetes were alive). The water of oligochaetes was changed every week. Worms collected from site A were observed at IATS for 10 days. A group of 30 remaining oligochaetes was subsequently transported to Hungary for further study. During the three-day transportation period, water temperature reached 25 °C. Upon arrival, the samples were examined on a slide under an Olympus research microscope at high magnification and the observed actinospores were recorded on videotape. Subsequently, digitised still images of the spores were taken from the video recordings with the help of a video image program (Imago®). Later on, drawings of the actinospores were made and their measurements were taken, as described by Lom et al. (1997).

Some of the worms observed and found infected on the cell-well plates were fixed in Bouin's solution for 4 h, dehydrated through a graded ethanol series, and embedded in paraffin or in Technovit 7100 acrylic resin (Kulzer, Heraeus, Germany). Sections from the two types of blocks were stained with haematoxylin & eosin or toluidine blue, respectively.

Results

Study of polychaetes collected from site B

One hundred and sixty polychaetes were collected from site B. All of them belonged to the same non-identified species. These polychaetes, as most of the organisms living in those waters, are euryhaline, but they were more abundant at the end of the channel, where salinity was higher. During the study, the worms died gradually, and the longest time of survival was two weeks. No actinosporean release could be observed in the plates during that period.

Study of oligochaetes collected from site A

Sixty oligochaetes belonging to the genus *Branchiura sowerbyi* were collected from site A. Worms kept individually in wells did not release actinospores during the first week of the study. In the water of the group of 30 worms transported to Hungary, numerous actinospores belonging to the same type were detected. A total of six worms released actinospores of the same type, resulting in a 20% prevalence of infection.

Mature spores (Figs 1, 2 and 3) were styleless, composed of a spore body and 3 caudal processes. The spore body contains the sporoplasm with 64 infective cells and 3 polar capsules somewhat pyriform in longitudinal view. The caudal processes are relatively short and a nucleus is visible in each of them. The main spore dimensions are presented in Table 1. According to the morphometric characteristics the detected actinospores should be regarded as an aurantiactinospore type (Fig. 4).

Table 1

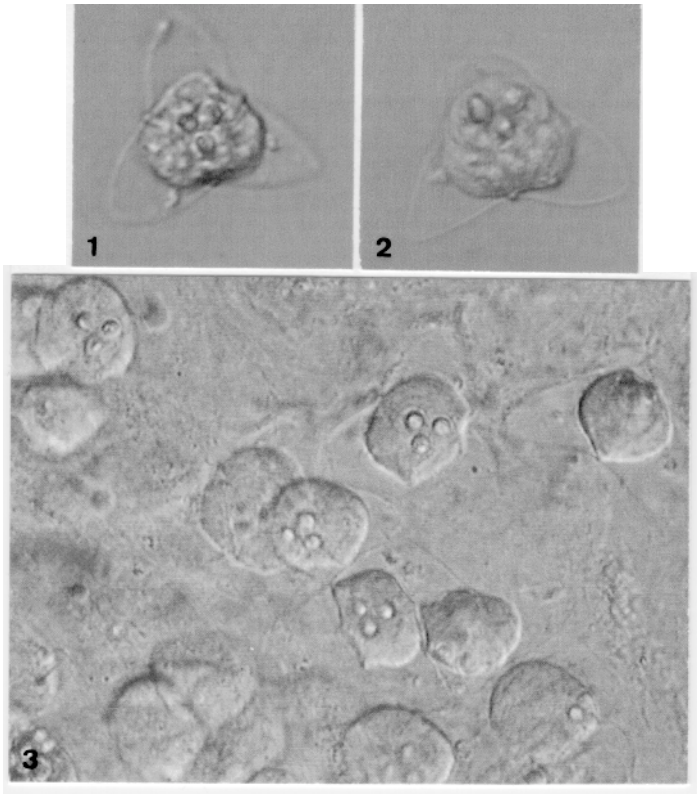
Spore measurements of the aurantiactinomyxon type detected during the survey (in μm)

Caudal process		Polar capsule		Spore body	Largest span
length mean (range)	width* mean (range)	width mean (range)	length mean (range)	diameter mean (range)	mean (range)
6.1 (4.8–7.3)	5.6 (4.8–6.5)	1.1 (1–1.1)	1.6 (1.5–1.7)	8.1 (7.3–8.9)	17.1 (16.1–18.5)

*measured near the sporoplasm

The histological study revealed the location of the parasite in the oligochaete. Extensive areas of the intestinal epithelium were invaded by parasitic stages (Figs 5–6), sometimes reaching the gut lumen (Fig. 7). Pansporocysts with spores in different maturing stages were the most common. Maturation seemed to occur from the inner part of the gut epithelium towards the lumen, as pansporocysts with developing stages were in the inner part of the gut (Figs 8–9), and fully mature spores were mainly closer to the lumen (Figs 5–7). In some sec-

tions, those mature spores were observed with the 3 polar capsules and the sporoplasm was filled with infective cells (Figs 10–11).



Figs 1 and 2. Waterborne aurantiactinospore from *Branchiura sowerbyi* collected from site A. Frontal view (1) and semi-lateral view (2). × 1900
Fig. 3. Aurantiactinospores released from an infected *Branchiura sowerbyi* under a coverslip. × 1500

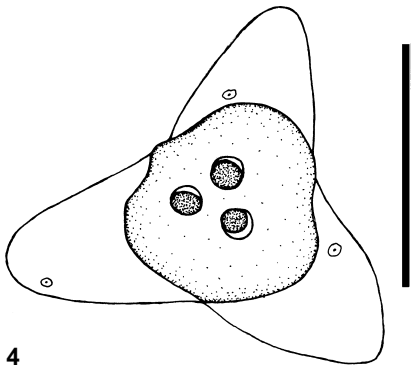
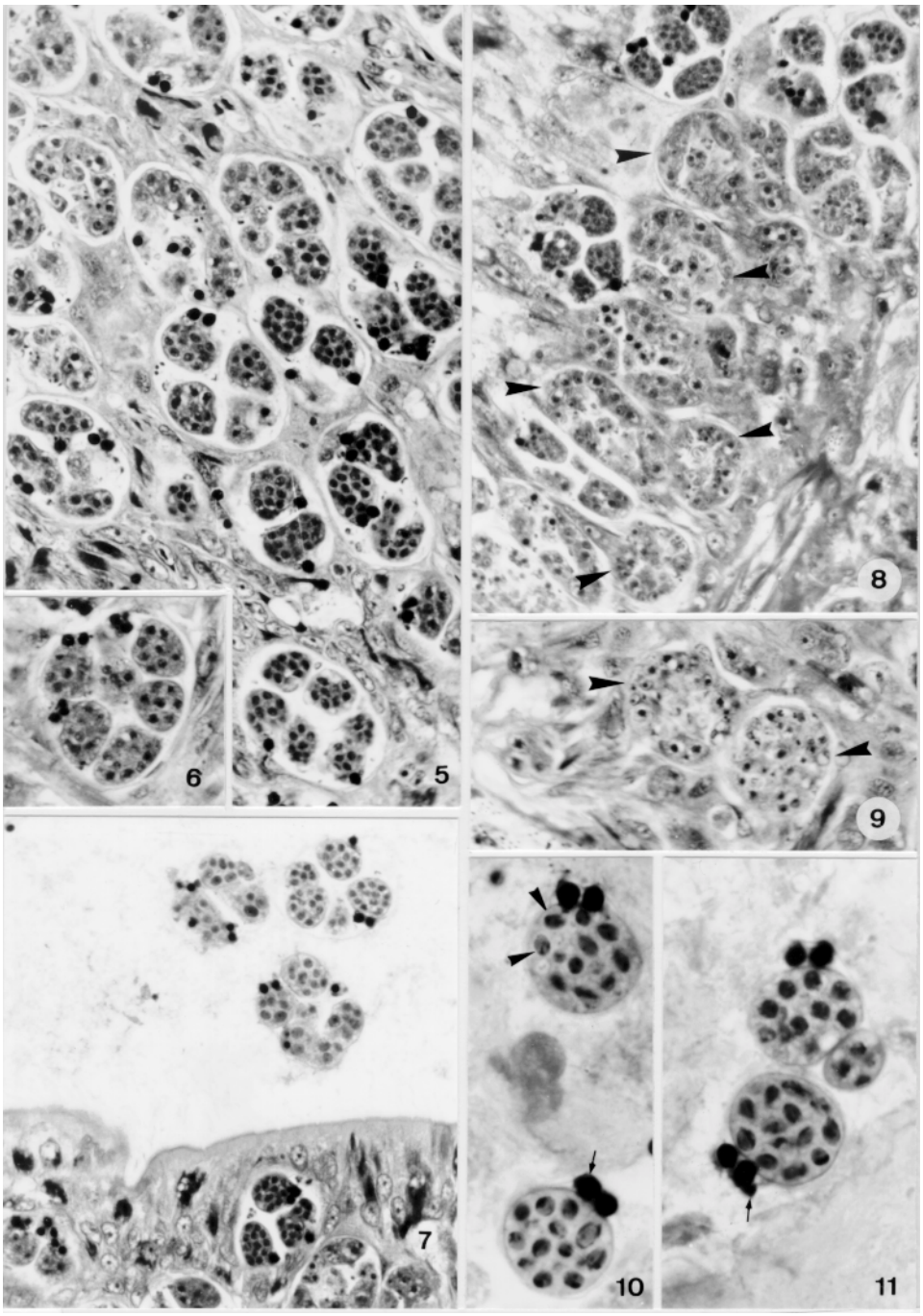


Fig. 4. Line drawing of the aurantiactinomyxon spore. Bar = 10 μm
Figs 5–11. Toluidine blue stained sections of *Branchiura sowerbyi* oligochaete infected by the aurantiactinomyxon. *Figs 5–6.* Intestinal epithelium heavily infected with mature pansporocysts (× 700). *Fig. 7.* Mature pansporocysts released into the gut lumen (× 500). *Figs 8–9.* Developing pansporocysts (arrowheads) in the inner part of the intestinal epithelium (× 500, × 700). *Figs 10–11.* Fully mature aurantiactinospores in the gut lumen. Infectious cells (arrowheads) and polar capsules (arrows) can be distinguished (× 1100)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Discussion

Actinosporean infections of oligochaetes from natural waters and fish ponds have been studied by many authors. In their relevant works, Ikeda (1912), Mackinnon and Adam (1924), and Janiszewska (1955, 1957) regarded Actinosporea exclusively as parasites of oligochaetes. After the revolutionary discovery of Wolf and Markiw (1984), different actinosporeans have been described as stages of myxosporea in alternate hosts (Hamilton and Canning, 1987; Székely, 1989; Burtle et al., 1991; Styer et al., 1992; Pote and Waterstrat, 1993; Yokoyama et al., 1993; Koller, 1994; Pallós, 1995; McGeorge et al., 1997; El-Mansy et al., 1998*a, b*; Xiao and Desser, 1998*a, b, c*).

Most of the above-listed authors reported a relatively low (about 1%) prevalence of infection in oligochaete populations. However, Yokoyama et al. (1993) detected actinosporean infection of more than 4% prevalence in *Branchiura sowerbyi* in goldfish-culturing ponds, El-Mansy et al. (1998*b*) recorded triactinomyxon infection of 33% prevalence in *Tubifex tubifex* in Lake Balaton, and aurantiactinomyxon infection of 59% prevalence was found in *Branchiura sowerbyi* from fish farms (El-Mansy et al., 1998*a*). The prevalence of infection found in the current study (20%) is clearly higher than that reported by most authors, but it should be regarded cautiously due to the preliminary approach of the sampling.

El-Matbouli et al. (1999) have described the effect of water temperature on the release of the triactinomyxon stage of *Myxobolus cerebralis*. In a similar way, the increase in water temperature during transportation could have contributed to actinosporean release, as the oligochaetes did not release actinospores while being kept at 15 °C in the present study.

The aurantiactinospore type detected in this study cannot be identified as any of the aurantiactinomyxon forms described by Marques (1984), Styer et al. (1992), Trouillier et al. (1996), McGeorge et al. (1997), Yokoyama (1997), or Xiao and Desser (1998*b*). As compared to their epispore, the processes of the aurantiactinomyxon types described by the above-mentioned authors are much longer than those of the aurantiactinomyxon type detected in this study, which has very short processes. In addition, the new type differs from all previously described aurantiactinomyxon types also in the number of secondary cells located in the sporoplasm. Furthermore, it cannot be compared with other aurantiactinosporean reports (El-Matbouli et al., 1992; Grossheider and Körting, 1992; Benajiba and Marques, 1993), in which spore dimensions were not recorded.

The aurantiactinospore detected in this study greatly resembles the aurantiactinomyxon type 8 found by El-Mansy et al. (1998*a*). It is also close to the aurantiactinospore of *Thelohanellus nikolskii*, obtained experimentally in *Tubifex tubifex* (Székely et al., 1998). However, the aurantiactinospore reported here is much smaller than the latter, and the number of secondary cells in its sporoplasm is much higher (Table 1).

The determination of the possible myxosporean alternate form of the described aurantiactinospore poses a very difficult task, as even the alternate host species is unknown. Experimental infections of parasite-free specimens of the fish species living in the water reservoir would be needed for determining the alternate host fish species and the myxosporean stage. Thus far, several myxosporean species have been described from freshwater fish in Spain (Alvarez-Pellitero et al., 1983; Alvarez-Pellitero and González-Lanza, 1985; Peribáñez et al., 1997), but this is the first report on actinosporean stages found in Spanish inland waters.

The current study failed to find Actinosporea in the sampled brackish/marine habitats. Nevertheless, there is an increasing number of myxosporean parasites from Spanish marine fish, mainly from cultured species (Alvarez-Pellitero and Sitjà-Bobadilla, 1993*a, b*; Palenzuela et al., 1999; Sitjà-Bobadilla and Alvarez-Pellitero, 1993; Branson et al., 1999). Thus, we still have no data on the alternate stages of these parasites which could develop in unknown putative hosts.

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