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Description of a new actinosporean type from South African freshwaters

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ABSTRACT: Actinospore infection of oligochaetes collected from the mud of 2 freshwater biotopes in South Africa was studied. Using the 'cell-well plate method', a new aurantiactinomyxon type was found in 1.1% of the examined *Branchiura sowerbyi* oligochaete specimens from the Rietvlei River, north of Johannesburg, Gauteng, South Africa. In 1.5% of *B. sowerbyi* collected in a pond (Padda Dam), near the Rand Afrikaans University, Johannesburg, the same aurantiactinomyxon type was found. Infected oligochaetes were found only after collection and no actinosporean release was recorded in *Branchiura* specimens kept alive for several weeks. Actinospore infection showed a high intensity in oligochaetes in both positive cases. Until now, no actinosporean stages of myxosporeans have been described from South Africa. The aurantiactinospore type presented in this communication differs from the known types already described in the literature.

KEY WORDS: Myxosporea · Aurantiactinomyxon · Actinospore · *Branchiura sowerbyi* · Oligochaete · Alternate host · Padda Dam · Gauteng · Rietvlei River · South Africa

INTRODUCTION

The first report on actinospores described these organisms as parasites related to myxosporeans (Štolc 1899). For a long time, this group of parasites was believed to represent an independent taxonomic entity. Research on actinosporeans became more intensive after Wolf & Markiw (1984) proved that they corresponded to the intraoligochaete developmental stages of fish-parasitic myxosporeans (Kent et al. 2001). The relevant research includes earlier surveys (Janiszewska 1957, Ormieres & Frezil 1969, Marques 1984) as well as studies of actinospore infection of oligochaetes in natural waters and fish farms in connection with the life cycle of myxosporeans (Kent et al. 2001). Relatively few data are available on myxosporean infections of African freshwater fish (Fantham 1930, Fomena & Bouix 1994, Reed 2002) and only a single paper appeared in Africa on their actinosporean alternative stages infecting oligochaetes (El-Mansy 2001).

Here, a new aurantiactinosporean stage of myxosporeans is reported from an oligochaete host from 2 freshwater biotopes in South Africa.

MATERIALS AND METHODS

Survey. The survey was conducted in 2 biotopes: (1) At 3 sampling points (Sites 1, 2 and 3) of the Rietvlei River, north of Johannesburg, where mainly *Clarias gariepinus* (Burchell, 1822) and *Barbus polylepis* Boulenger, 1970 are found. Other abundant fish species living in the river are *Cyprinus carpio* Linné, *Tilapia sparrmanii* Smith, 1840, *Pseudocrenilabrus philander* (Weber, 1897). (2) In a pond (Padda Dam), near the Rand Afrikaans University, Johannesburg, where the most abundant fish were *Aplocheilichthys johnstoni* Günther, 1893, *P. philander* (Weber, 1897), *T. sparrmanii* Smith, 1840, and *Clarias gariepinus* (Burchell, 1822), and the introduced *C. carpio* Linné is also abundant. All sampling was conducted in the second half of September 2003 before the rainy summer season at a water temperature of 20°C. To detect the intraoligochaete actinospore types, mud samples were collected and transported to the fish parasitology laboratory of the Rand Afrikaans University. The oligochaetes were washed out from the mud with a net and gently picked up by forceps. After collection, the oligochaetes were individually placed into wells of

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cell-well plates (according to Yokoyama et al. 1991). Starting the following day, the water layer above each oligochaete in each of the wells was examined daily for 3 d for the presence of released actinospores. Then, the oligochaetes were kept in plastic tubes for 1 wk and the water in each tube was changed daily in order to keep the oligochaetes alive. The oligochaetes were then transported to Hungary and monitored for actinospore release for an additional 2 mo.

If examination with a stereomicroscope indicated the presence of spores, the actinospores floating or adhering to the walls of the cell-well plates were examined on a slide with a light microscope at higher magnification. Microphotographs were then taken of the spores using a Zeiss Axioplan 2 compound microscope attached to a computer and the digital pictures were archived by Axiovision software. Subsequently, drawings were made of the actinospores and their measurements were taken. The characteristic dimensions of actinospores (size of polar capsules, diameter of spore body, length and width of caudal processes, largest span of processes) and the number of secondary cells in the sporoplasm were recorded by measuring newly released spores according to the guidelines suggested by Lom et al. (1997). To determine the dimensions of actinospores, measurements of 10 mature spores were averaged. A part of the released actinospores were fixed in 80% ethanol and transported to Hungary for further morphological studies. Measurements of spores were calculated from specimens on digitised photographs in South Africa and some additional measurements were made on 20 actinospore specimens fixed in ethanol. The measurements of these latter spores were measured with the help of a WinImago® program. Some of the representative oligochaetes from all the examined biotopes were fixed in 80% ethanol and identified in Estonia as described by Timm (1997).

For histological sections, infected oligochaetes were fixed in 10% neutral buffered formalin and embedded in paraplast-wax. Five µm thick sections were made and stained with haematoxylin and eosin. Photomicrographs of the sections were taken with an Olympus DH-10 digital camera mounted on an Olympus BH2 microscope.

RESULTS

Survey

Samples sent for identification and representing all of the 4 collection sites proved to contain developed and juvenile forms of *Branchiura sowerbyi*. Of the 93 examined oligochaetes from the 3 stretches of the Rietvlei River, only 1 large-sized *B. sowerbyi* specimen (1.1%) collected from Site 3 proved to be infected with a new aurantiactinomyxon type (Fig. 1). Another actinosporean release was found in a single specimen of the 65 *B. sowerbyi* oligochaetes (1.5%) collected from Padda Dam (Fig. 2). In both cases, aurantiactinomyxon types were released. All the spores were released during the first day, after collection of the oligochaetes from the mud, into the wells of the cell-well plate. No further actinospore release was found in other oligochaete specimens from the same biotope during the 2 mo observation period.

Effect of ethanol fixation on the secondary cells of the sporoplasm

Secondary cells in sporoplasms of actinospores were not distinguishable in living aurantiactinospores (Fig. 3). However, in spores fixed in 80% ethanol and examined 1 mo later, contours of the secondary cells became well defined and the number of secondary cells as well as the polar capsules could be exactly counted (Fig. 4).

Histology

Early developmental stages and pansporocysts were located in the intestinal epithelium of the oligochaete, deforming and enlarging the normally monolayered epithelium (Fig. 5). At the infected part of the gut, almost each epithelial cell contained pansporocysts of different developmental stages. In some of the pansporocysts, the 8 developing actinospores were clearly visible. In the more developed pansporocysts, however, 4 actinospores were cross-sectioned at the level of the sporoplasm and revealed the presence of secondary cells. Uninfected epithelial cells were encountered only in small stretches around the intestinal lumen of the worms. In other parts of the degenerated epithelium, nuclei of the deformed epithelial cells were jammed between the spaces of pansporocysts (Fig. 6).

Description of the detected new actinospore type

From both infected *Branchiura* specimens of the 2 sampling sites, similar aurantiactinospores were released. Only minor non-significant differences were observed, both in terms of the dimensions and form of the spores. This aurantiactinomyxon type, which differs from the already known actinospores, has been described as follows on the basis of spores collected from the *Branchiura* specimen from Padda Dam

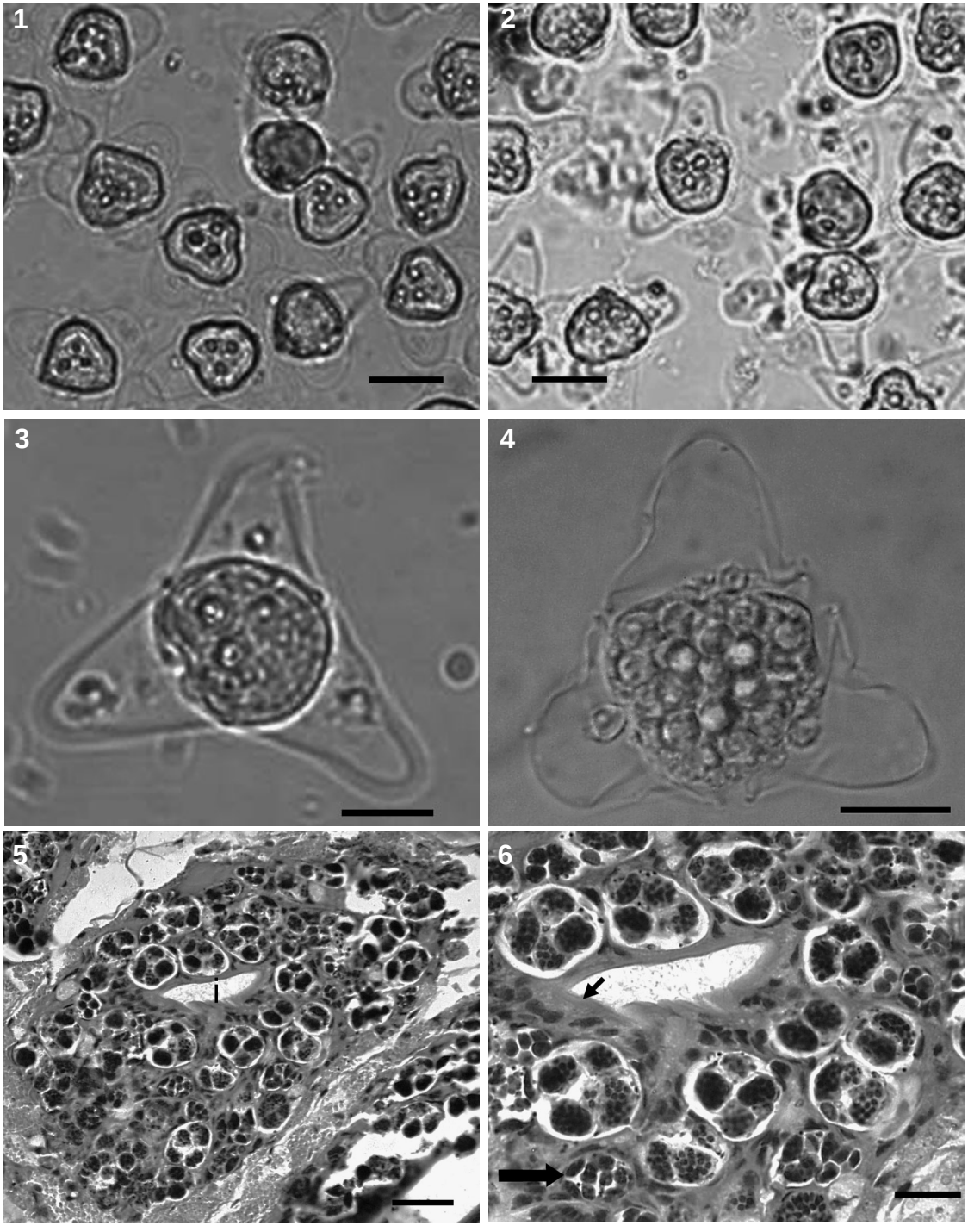

Fig. 1. Aurantiactinospores released from a *Branchiura sowerbyi* specimen collected from the Rietvlei River. Scale bar = 20 µm. Fig. 2. Aurantiactinospores released from a *B. sowerbyi* specimen collected at Padda Dam. Scale bar = 20 µm. Fig. 3. Enlarged picture of a freshly released aurantiactinomyxon. In the seemingly homogenic mass of the sporoplasm, only the 3 polar capsules are seen. The nuclei of the caudal projections can also be seen. Scale bar = 10 µm. Fig. 4. Aurantiactinospores fixed in 80% ethanol. Besides nuclei of the caudal projections, secondary cells of the sporoplasm are also seen. Scale bar = 10 µm. Fig. 5. Histological section of a *B. sowerbyi* oligochaete. In each segment, jammed cells of the proliferated epithelium containing aurantiactinomyxon developmental stages are located around the intestinal lumen (i). H & E. Scale bar = 15 µm. Fig. 6. Enlarged part of Fig. 5. Remnants of the relatively unaffected epithelial cells are found only in a short stretch around the intestinal lumen (small arrow). Most of the epithelial cells are infected with pansporocysts of an aurantiactinomyxon type. In younger pansporocysts (large arrow), 8 developing spores can be detected. In more developed pansporocysts, only 4 spores locating in 1 level and containing secondary cells are seen. H & E. Scale bar = 30 µm

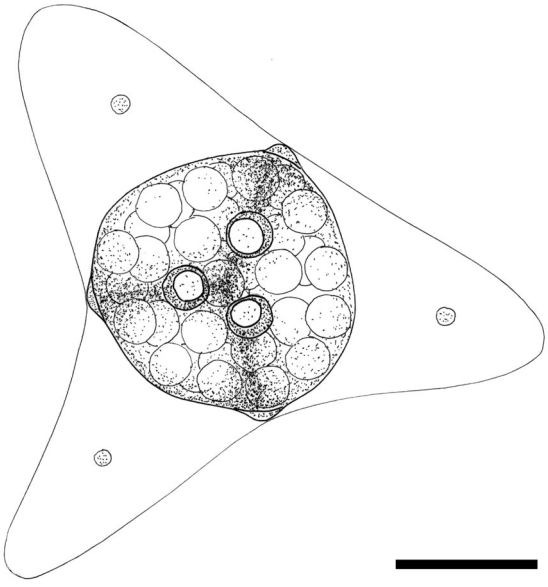

Fig. 7. Schematic drawing of the aurantiactinospore type released by a *Branchiura sowerbyi* specimen from Padda Dam. Scale bar = 10 µm

(Fig. 7). Minor variations observed in morphology and size between 2 oligochaetes are presented in Table 1.

Aurantiactinomyxon type
(Figs. 2 & 7, Table 1: Type 2).

Spores always single, epispore without style with 3 leaf-like short caudal processes of equal length. Spores released from *Branchiura sowerbyi*. Spore body 19.6 µm (18.1 to 21.8) in diameter with 3 apical polar capsules 2.7 µm (2.6 to 2.9) in diameter. Secondary cells, 64. Three caudal processes 10.5 µm (9.9 to 17.4) long and 15.2 µm (13.3 to 18.7) wide.

DISCUSSION

Aurantiactinomyxon types as such or as the alternative spore forms of myxosporeans have been described by the authors listed in Table 1. The aurantiactinomyxon type detected in this study differs substantially from the types already described in at least 1 of its dimensions.

Based on the measurements described in Table 1, the aurantiactinospores detected in this study, although collected from 2 different biotopes, appear to be identical and represent a new form hitherto not described.

The aurantiactinomyxon type found during this survey differs from the over 40 known types not only in its measurements and shape, but in the majority of cases also by the oligochaete alternate hosts. Until now, only Yokoyama et al. (1993), Yokoyama (1997), El-Mansy et al. (1998a,b) and Székely et al. (2000) have recorded aurantiactinomyxon types of the relatively large-sized *Branchiura sowerbyi.* The prevalence of actinosporean infection was relatively low, significantly less than found by El-Mansy et al. (1998b) in Lake Balaton or by Székely et al. (2003) in Japan, but corresponded to results obtained in other similar surveys (Xiao & Desser 1998, Negredo & Mulcachy 2001, Özer et al. 2002 and Oumona et al. 2003). The low prevalence only represents a temporary situation as Gilbert & Granath (2001) proved that an oligochaete can repeatedly release actinospores after a long dormitory period. Furthermore, our unpublished observations prove that *Branchiura* specimens brought to the laboratory from natural waters preserve their infectiveness and that even without re-infection, they are able to release actinosporeans 18 to 24 mo after being collected. It is not excluded, therefore, that *Branchiura* specimens brought from South Africa and kept alive in Hungary might produce auroactinospores or other actinospores in the future.

Experts in the systematics of myxosporeans disagree concerning the description of new actinospore types detected from oligochaetes. According to Kent & Lom (1999), actinospores detected from oligochaetes should be regarded as developmental stages of Myxozoa species parasitising vertebrate hosts, primarily fish, whereas Lester et al. (1998) are of the opinion that new species of Myxozoa can be described based solely on the actinospores. Considering their arguments, we accept the guidelines provided by Kent & Lom (1999) and describe new actinospores only as types in every case. Their description as species should be based on the production of myxospores, experimentally obtained in the vertebrate host or on their identification by molecular biological methods.

When describing actinosporean types, one has to be especially careful. Morphological characterization of the forms found is extremely important but in itself not enough. One cannot know whether the shape of the actinospores is as stable as in the case of myxospores. Hallett et al. (2002) stated that the molecular patterns of 2 morphologically clearly differing actinosporean types proved to be genetically identical. On the other hand, we cannot be sure that 2 morphologically similar actinosporean types, with identical dimensions, would be identical at the molecular biological level.

In the identification and description of actinosporean stages, the number of secondary cells in the sporoplasm is a very important key. These numbers are usually multiples of 2 (4, 8, 16, 32, etc.). Unfortunately, counting the numbers of these cells is not easy; therefore, for most of the described actinosporean types, this detail was not provided or only an approximate number was given. In fresh spores, the structure of the

Table 1. Comparison of measurements of aurantiactinomyxon type spores detected during the present study and those found in the scientific literature. nd: not determined; l.: length; w.: width (µm)

Source	Locality	Aurantiactinomyxon type/species or myxosporean form/fish host	Length of caudal process (range); width of caudal process at the basis (range)	Polar capsule l. (range); w. (range)	Diameter of spore body (range)	Largest span (range)	No. secondary cells	Alternate host
Present study	South Africa, Rietvlei River	Type 1	10.4 (9.8–16.9) 15.0 (13.1–18.2)	2.7 (2.6–2.8)	19.5 (17.9–21.4)	36.7 (32–39.2)	nd	*Branchiura sowerbyi*
Present study	South Africa, Padda Dam Johannesburg	Type 2	10.5 (9.9–17.4) 15.2 (13.3–18.7)	2.7 (2.6–2.9)	19.6 (18.1–21.8)	35.8 (30.2–41.3)	64	*B. sowerbyi*
Janiszewska (1957)	Poland, River Ropa	*A. raabei iunioris*	25–30 nd	nd	17	nd	16	*Limnodrilus hoffmeisteri*
Ormières (1968)	France?	*A. pavinsis*	12 (10–14) nd	3 (2.5–3.5) 2 (1.5–2.5)	10 (9–11)	nd	12	*Stylodrylus heringianus*
Ormières & Frézil (1969)	Yugoslavia, Lake Ochrid	*A. eiseniellae*	10–15 nd	nd	12	nd	32	*Eiseniella tetraedra*
Marques (1984)	France	*A. pavinsis*	10–12 (15–20)	nd	8 (12)	nd	16	*Stylodrylus heringianus*
Marques (1984)	France	*A. stellans*	70–90	nd	15–20	nd	16	*Tubifex* spp.
Marques (1984)	France	*A. trifolium*	40–50	nd	20–25	nd	32	*Tubifex* spp.
Burtle et al. (1991)	USA, catfish pond	Aurantiactinomyxon/ PGD organism/ channel catfish	29.5 nd	nd	19.5	nd	>32	*Dero digitata*
Bartholomew et al. (1992)	USA, experimental tanks	Type 1	nd	nd	nd	nd	nd	*Nais bretscheri*
El-Matbouli et al. (1992)	Germany, experimental infection	*Hofferellus carassii* Goldfish	(25) nd	nd	(13)	nd	nd	Mixed species: *T. tubifex*, *T. ignotus* and *L. hoffmeisteri*
Grossheider & Körting (1992)	Germany, experimental infection	*H. cyprini* Common carp	(30) nd	nd	(15)	nd	nd	*Nais* spp.
Styer et al. (1991)	USA, experimental infection	*A. major* PGD/ channel catfish	11 × 36	nd	18–22	nd	nd	*D. digitata*
Styer et al. (1991)	USA, experimental infection	*A. minor* PGD/ channel catfish	36	nd	13–16	nd	nd	*D. digitata*
Benajiba & Marques (1993)	France, experimental infection in tanks	*Myxidium giardi* European eel	(20) nd	nd	(12)	nd	nd	*Tubifex* spp.
Pote & Waterstrat (1993)	USA (Mississippi State), Fish Pond	PGD organism/ channel catfish *Ictalurus punctatus*	26 (21–32) 11 (8–12)	nd	23 (20–24)	nd	nd	*D. digitata*
Trouillier et al. (1996)	Germany, experimental tank	Aurantiactinomyxon/ *H. carassii*/ goldfish	48.8 (40.6–57) 11.7 (10.2–13.3)	nd	23.5 (20.4–26.6)	nd	nd	*Nais elinguis*
Yokoyama (1993)	Japan, goldfish pond	Aurantiactinomyxon spp. 1	16 nd	nd	11	nd	8	*B. sowerbyi*
McGeorge et al. (1997)	Scotland, Salmon hatchery stream	Aurantiactinomyxon/ *Sphaerospora truttae*	25.6 (19–31) nd	2.7 (2–3)	13.7 (12–15)	nd	nd	Immature tubificid
Yokoyama (1997)	Japan	*Thelohanellus hovorkai* Common carp (koi)	25–33 nd	nd	18–22	nd	32	*B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 1	17.5	2 × 2	18.3	45.4	nd	*T. tubifex*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 2	65.7 10.5	4 × 1.7	22.8	142.5	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 3	70.3 8	2.9 × 2.9	22.8	149.3	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 4	55.7 11.2	2.9 × 2.9	19.4	122	nd	Water, *B. sowerbyi*

(Table continued on next page)

Table 1 (continued)

Source	Locality	Aurantiactinomyxon type/species or myxosporean form/fish host	Length of caudal process (range); width of caudal process at the basis (range)	Polar capsule l. (range); w. (range)	Diameter of spore body (range)	Largest span (range)	No. secondary cells	Alternate host
El-Mansy et al. (1998a)	Hungary, fish pond	NO 5	17.2 3.9	1.4 × 1.4	9.9	39.5	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 6	24.2 11.2	2.8 × 2.8	19.7	55.6	nd	*Limnodrilus* spp.
El-Mansy et al. (1998a)	Hungary, fish pond	NO 7	24.4 9.5	2.8 × 2.5	18.9	58.4	nd	Water
El-Mansy et al. (1998a)	Hungary, fish pond	NO 8	12.2 9	1.4 × 1.4	22.6	39.8	nd	*Limnodrilus* spp.
El-Mansy et al. (1998a)	Hungary, fish pond	NO 9	51.3 9.5	2.3 × 2.3	18.8	103.2	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 10	16.7 8.8	1.7 × 1.7	15.5	39.5	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998a)	Hungary, fish pond	NO 11	31.9 3.7	3.4 × 2	8.5	46.5	nd	Water
El-Mansy et al. (1998a)	Hungary, fish pond	NO 12	26.5 8.7	2.8 × 3.1	12.1	59.2	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998b)	Hungary, Lake Balaton	NO 1	51.3 9.5	2.3 × 2.3	18.8	103.2	nd	Water, *B. sowerbyi*
El-Mansy et al. (1998b)	Hungary, Lake Balaton	NO 2	22.5 11.7	2.8 × 2	21.1	52.2	nd	*Limnodrilus*
El-Mansy et al. (1998b)	Hungary, Lake Balaton	NO 3	17.2 3.9	1.4 × 1.4	9.9	39.5	nd	Water, *B. sowerbyi*
Székely et al. (1998)	Hungary, experimental infection	*T. nikolskii*/ common carp	13.4 (11.3–15.5); 9 (8.5–9.6)	2.1 (2–2.2); 2.1 (2–2.2)	21.1 (21–21.2)	40.5 (40–41)	16	*T. tubifex*
Székely et al. (1998)	Hungary, experimental infection	*T. hovorkai*/ Common carp	29 (28.2–29.6); 9.2 (8.1–10.2)	3.42 (3.4–3.5); 3.36 (3.3–3.4)	18.6 (18.3–18.9)	65.2 (65.1–65.3)	32	*B. sowerbyi*
Xiao & Desser (1998)	Canada, Lake Sasajewun	Type 1	24 (21–26); 13–16	3 (2.7–3.4); 1.5 (1.4–1.7)	12 (11.5–13.8); 11 (10–12.5)	nd	64–128	*L. hoffmeisteri*
Lin et al. (1999)	USA, catfish pond	Aurantiactinomyxon janisewskai/*Henneguya exilis*/channel catfish	nd	nd	nd	nd	nd	*D. digitata*
Székely et al. (2000)	Spain, Mijares River/ Sitjar Reservoir	Type 1	6.1 (4.8–7.3); 5.6 (4.8–6.5)	1.6 (1.5–1.7); 1.1 (1–1.1)	8.1 (7.3–8.9)	17.1 (16.1–18.5)	64	*B. sowerbyi*
Negredo & Mulcachy (2001)	Ireland, Cloonee River	Aurantiactino-myxon 1	21.1 (18.2–23.4) 16.1 (13–19.5)	3 (2.5–3.9)	14.4 (12.6–16.9)	nd	nd	*T. ignotus*
Negredo & Mulcachy (2001)	Ireland, Cloonee River	Aurantiactino-myxon 2	31 (26–36) 10.6 (9.1–13)	2.7 (2.6–3.1)	14.1 (13–15.6)	nd	nd	*L. hoffmeisteri*
Negredo & Mulcachy (2001)	Ireland, Cloonee River	Aurantiactino-myxon 3	20.8 (18.2–23.4) 10.4 (10.4–10.4)	nd	9.1 (9.1–9.1)	nd	nd	*T. ignotus*
Hallett et al. (2002)	Germany, Bavaria	Auratiactino-myxon morphotype 1	37.3 (28.5–49.2) 15.7 (14.2–18.1)	3.1 (2.6–3.9)	19.4 (16.8–21.4)	82.4 (68.6–103.6)	30	*T. tubifex*
Hallett et al. (2002)	Germany, Bavaria	Auratiactino-myxon morphotype 2	87.7 (75.1–103.6) 13.1 (10.4–15.5)	3.1	19.7 (18.1–22.0)	163.7 (134.7–194.3)	30	*T. tubifex*
Özer et al. (2002)	Scotland, salmon farm	Type 1	32 (31–36)	nd	14.4 (12–15)	nd	64–128	*T. tubifex*
Özer et al. (2002)	Scotland, salmon farm	Type 2	24.7 (23.6–26.5)	nd	14.9 (14–18.7)	nd	64	*T. tubifex*
Özer et al. (2002)	Scotland, salmon farm	Type 3	114.5 (101.4–124.8)	nd	24 (23.4–24.9) 21.8 (20.3–23.4)	nd	32	*T. tubifex*
Özer et al. (2002)	Scotland, salmon farm	Type 4	28.3 (23.4–31.2)	nd	11.9 (11.2–14)	nd	32	*T. tubifex*
Oumuna et al. (2003)	Germany, Bavarian fish farm	*A. pavinsis*	12 (10–14) nd	3 (2.5–3.5) 2 (1.5–2.5)	10 (9–11)	nd	12	Water *T. tubifex?*
Oumuna et al. (2003)	Germany, Bavarian Fish Farm	Aurantiactino-myxon type 1	76 (75–77)	5 (4.7–5.3) 4 (3.8–4.2)	16 (15–17)	nd	nd	Water
Székely et al. (2003)	Japan, brook on Fuji Mountain near Yamanashi	Type 1 13.5 (13–14)	12.4 (10–14) 1	2	13.5 (13–14)	26.8 (25–30)	8	*T. tubifex*

sporoplasm is hardly seen, therefore the number of secondary cells was counted either by compressing the spore body (Székely et al. 1998, Molnár et al. 1999) or in histological sections (Alvarez-Pellitero et al. 2002). The observation that alcohol-preserved specimens reveal the contours of secondary cells well, might prove to be a new method for specialists working on actinosporeans.

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