(57) ***BORKHANUDDIN H., OSTOROS GY, MOLNÁR K., SZÉKELY CS.***

New data on the actinosporean stages of fish-parasitic myxosporeans in oligochaete alternative hosts in Lake Balaton in 2011

Új adatok a halparazita nyálkaspórások oligochaetákban fejlődő alternatív aktinospóra stádiumairól a Balatonban (2011)

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, 1143. Budapest. Hungária krt. 21.

Introduction

Even though numerous identification and taxonomic study conducted on myxozoans, interest in this group has intensified along the development of fisheries aquaculture for their significant impact or risks on the cultured fish (Morris & Freeman, 2010). The myxozoan life cycle involved two distinctive parasitic stages namely myxospore (infecting the fish hosts) and actinospore (infecting the annelid hosts). In Hungary, intensive works on myxozoans have been done by some of the senior authors (Molnár and Székely) in the 90's until recent time (El-Mansy et al., 1998a, and Kelemen et al., 2009). Authors of the above papers in their studies collected several actinospore types from Lake Balaton in Hungary. The work presented here is a continuing collection of the actinospore stages in Lake Balaton, Hungary.

Materials and methods

Actinospores were collected from the infected oligochaetes. Oligochaetes were sampled in five sampling points of the Lake Balaton (Table 1). A total of 3528 individuals of oligochaetes were isolated and studied in a "cell-well plate" by routine examination during the study time (April 2011- August 2011). The main, so-called "large sized" oligochaete species collected was *Isochaetides michaelseni* (Lastočkin, 1937). An intensive monitoring was also conducted onto the less studied "small oligochaetes" to determine the feasibility of these worms for actinospore infection. The majority of the "small sized" oligochaetes belonged to *Nais* and *Dero* spp. In addition to isolation works routine observations were conducted for monitoring the length of time interval to produce new spores clutch by the infected oligochaetes, as well as for getting data on the number of spores released at each interval. Monitoring has been still in progress.

Results and discussion

In total, 3528 "large" oligochaetes, *I. michaelseni* were collected; 63% of the worms were collected from Keszthely (2232 individuals). Seven types of actinospores were found namely, Aurantiactinomyxon (AAM – 2 types), Echinactinomyxon (ECM – 1 type), Raabeia (RB – 1 type), and Triactinomyxon (TAM – 3 types). Triactinomyxon (TAM) types of actinospores were most frequently released by the worms, and of them the triactinomyxon stage (TAM) of *Myxobolus pseudodispar* seems to be the most dominant actinospore.

Table 1: *No. of collected & no. of actinospores infected according to location*

Location	Annelid (Oligochaete)	No. of Oligochaetes Observed	No. of Oligochaete Infected
Keszthely	*I. michaelseni*	2232	67 (49-TAM, 11-AAM, 4-RB, 3-ECM)
Tihany	*I. michaelseni*	288	24 (16-TAM, 8-AAM)
Balatonszemes	*I. michaelseni*	96	9 (7-TAM, 2-AAM)
Siófok	*I. michaelseni*	838	20 (19-TAM, 1-AAM)
Balatonvilágos	*I. michaelseni*	74	0

Table 2: *Prevalence (in percentage, %) of different actinospores in oligochaetes from Lake Balaton, collected in 2011*

	Prevalence (%)			
Location	Triactinomyxon	Aurantiactinomyxon	Raabeia	Echinactinomyxon
Keszthely	2.2	0.4	0.1	0.1
Tihany	5.5	2.8	/	/
Balatonszemes	7.3	2.0	/	/
Siófok	2.4	0.1	/	/
Balatonvilágos	/	/	/	/

Table 3: *Infection prevalence (%) for each actinosporean collective group*

Actinospore group	No. of Oligochaetes Infected	Prevalence (%)
Triactinomyxon	91	2.5
Aurantiactinomyxon	22	0.6
Raabeia	4	0.1
Echinactinomyxon	3	0.08

Prevalence rate of actinosporean infections

Of the 3528 oligochaetes monitored from the Lake Balaton, only 120 worms (3.4%) from 4 locations (Keszthely, Tihany, Balatonszemes, and Siófok) were infected by actinospores. Actinospores were absent in oligochaetes examined from Balatonvilágos.

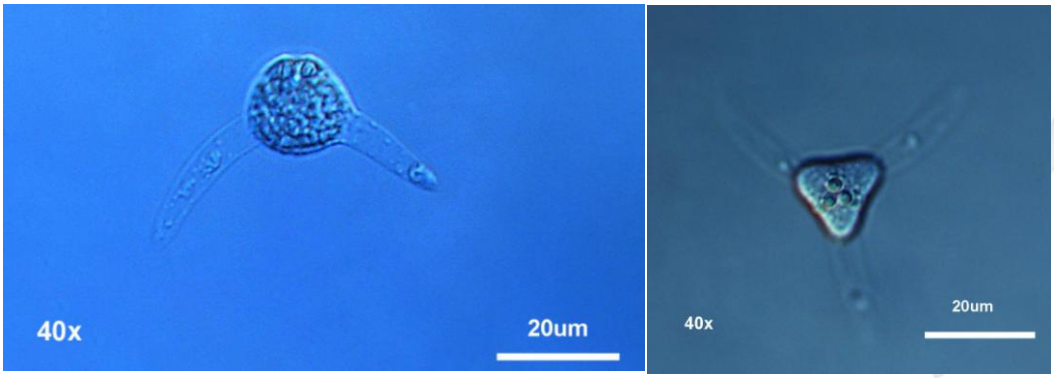
In the case of individual actinosporean groups at particular sampling location, prevalence of the triactinomyxon in Keszthely, Tihany, Balatonszemes and Siófok, were 2.2%, 5.5%, 7.3% and 2.4% respectively. Aurantiactinomyxon prevalence was highest (2.8%) in Tihany and lowest (0.1%) in Siófok. Raabeia and Echinactinomyxon were only found in Keszthely with 0.1% prevalence (Table 2). Overall, the collective triactinomyxon group was the most common infecting 91 oligochaetes (2.5% prevalence). Other collective groups were the least common showing prevalence in between 0.08 – 0.6% (Table 3).

Actinospores Collective Groups

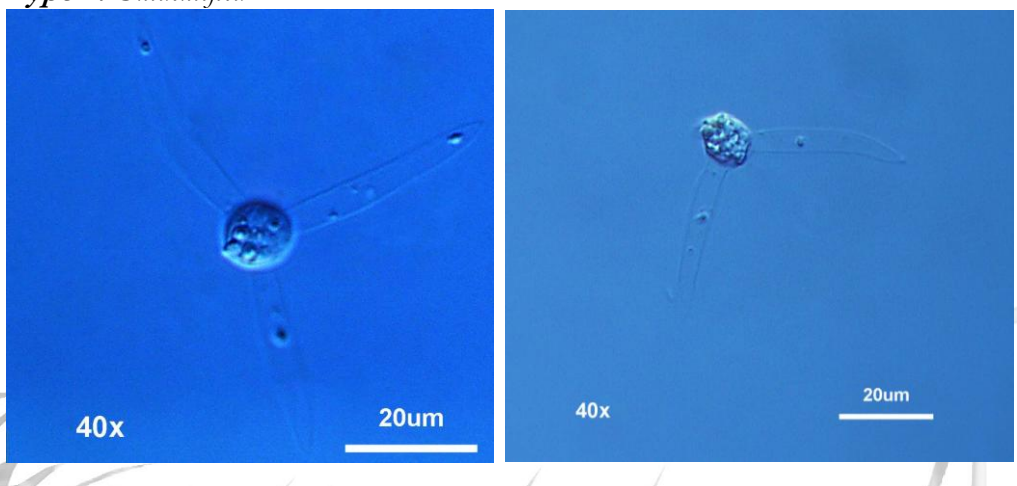
Actinospore types belonging to four collective groups were collected in the present study; namely: aurantiactinomyxon (2 types), echinactinomyxon (1 type), raabeia (1 type), and triactinomyxon (3 types). Figures of the spores are as follows:

Aurantiactinomyxon Janiszewska, 1952

Type 1: *Unidentified*

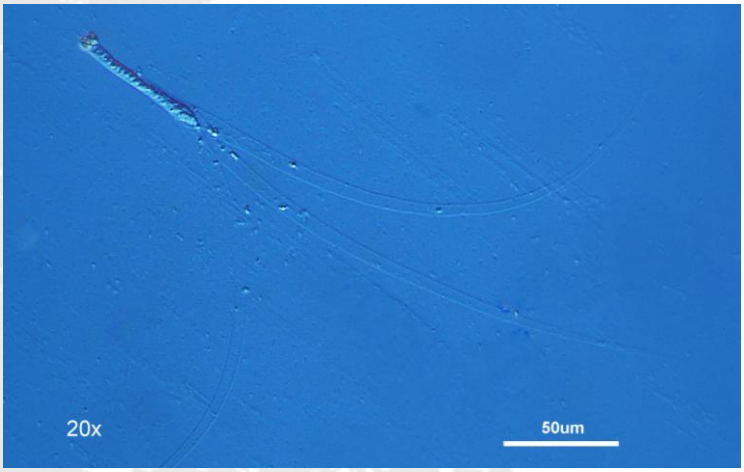


Type 2: *Unidentified*



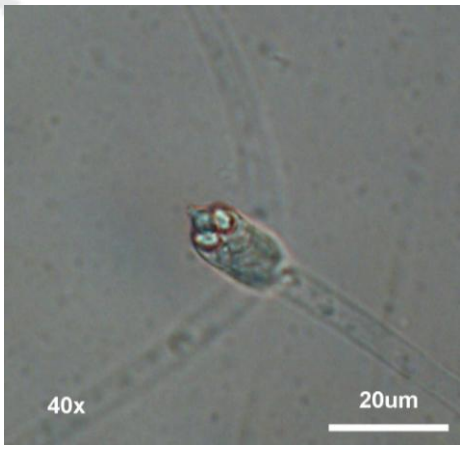
Echinactinomyxon Janiszewska, 1957

Type 1: *Unidentified*



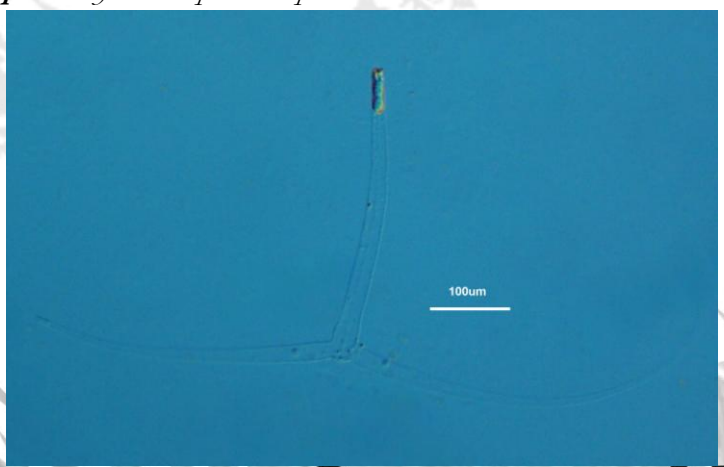
Raabeia Janiszewska, 1955

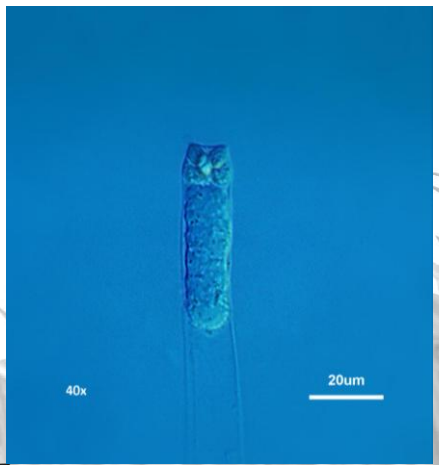
Type 1: *Unidentified*



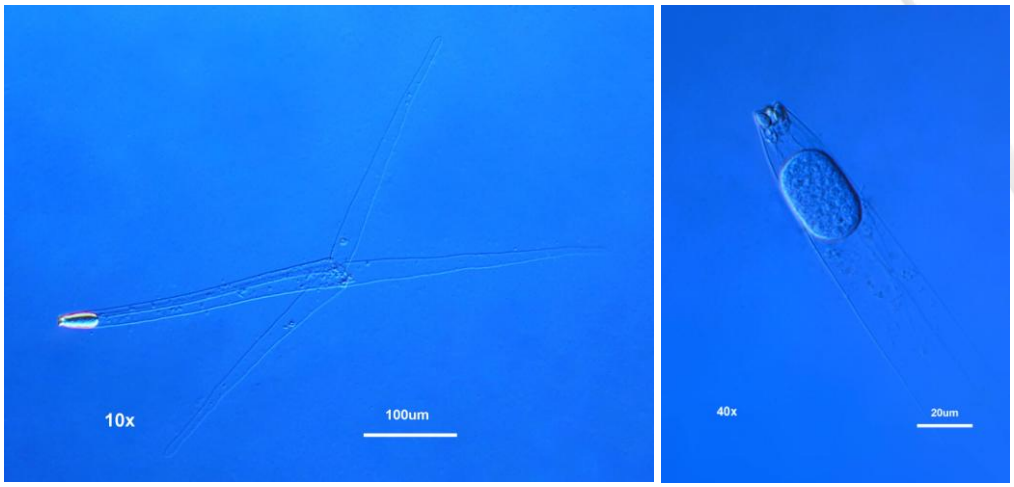
Triactinomyxon Štolc, 1899

Type 1: *Myxobolus pseudodispar*

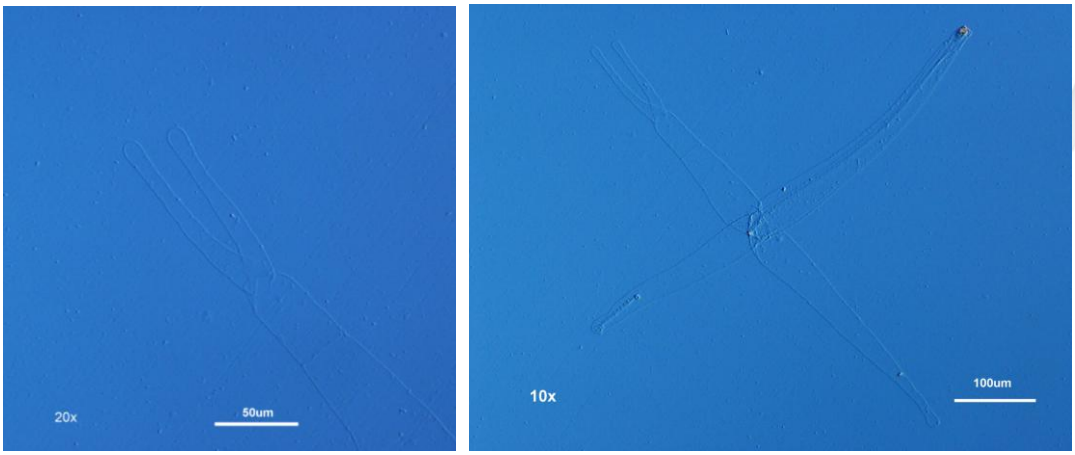




Type 2: *Myxobolus shaharomae*



Type 3: *Unidentified*



Discussion

It is estimated that 136 types of actinospores belonging to different collective groups have been described recently. Apart from that, some actinospore genera and types were still established as independent zoological categories (Lom & Dykova, 2006).

In between 1997 and 1998, El-Mansy et al. (1998a) has collected 10 types of actinospores belonging to three main groups namely triactinomyxon (5 types), raabeia (2 types), and aurantiactinomyxon (3 types), while Kelemen et al. (2009) have isolated 13 morphologically distinct actinospore types (5 triactinomyxons, 3 echinactinomyxons and 5 aurantiactinomyxons). On the other hand, our study between April and August of 2011 indicated 7 types of actinospores. In addition to the three groups previously described by El-Mansy et al., (1998a), we also found one new group which is the echinactinomyxon. From the studies, actinospores were identified through morphological and taxonomical perspective. The findings from El-Mansy et al. (1998a) and current study showed a relatively high diversity of the spores in Lake Balaton. It also indicates that after molecular studies some

of them might be identified as with alternative stages of pathogenic myxosporeans infecting Lake Balaton fishes or other vertebrates.

Prevalence rate of the actinospores infecting *I. michaelseni* from this study could be considered low with 3.4% prevalence. However, El-Mansy (1998a) recorded prevalence up to 33-34% from the oligochaetes (*T. tubifex* & *L. hoffmeisteri*) collected from Lake Balaton. It is believed that, the major differences of prevalence are due to the number of worms examined. In their study El-Mansy et al., (1998a) examined 1164 worms, while 3528 worms examined in the present study. Gregory & Blackburn (1991) stated that a confounding variable in studying parasites biology is the sample size where the number of individuals of a host species examined for parasites. Prevalence could be referred as the proportion of host individuals (in our case is the oligochaetes) in a sample that are infected with the parasites (actinospores) (Gregory & Blackburn, 1991). According to Özer et al., (2002), in some cases environmental conditions could differentiate the actinospore prevalence, on which a study was conducted. In the case of El-Mansy et al., (1998b), fish polyculture pond with a mud substrate might harbour large oligochaete population. With no flushing out of the released myxospores into the pond, chances of infections on the oligochaetes were higher compared to flowing water system.

Table 4: *Comparison of prevalence of actinospores collected during the present study and some previous studies.*

Locality	Annelid host	No. of Oligochaetes Collected	No. of Oligochaetes Infected	Prevalence (%)	References
Scotland, Atlantic Salmon farm	*T. tubifex, L. hoffmeisteri & Lumbriculus variegatus*	28, 387	823	2.9	Özer et al., 2002
Japan, Mena River	*Rhyacordrilus komarovi*	620	16	2.6	Székely et al., 2003
Japan, Chitose River	*Lumbriculus variegatus*	680	55	8.1	Székely et al., 2003
Japan, Fuji Mountain	*T. tubifex, Rhyacodrilus coccineus, Dero digitata, L. hoffmeisteri*	130	1	0.77	Székely et al., 2003
Hungary, Lake Balaton	*T. tubifex & L. hoffmeisteri*	1164	274	23.5	El-Mansy et al., 1998a
Hungary, Lake Balaton	*I. michaelseni*	3528	120	2.6	Present study

The work by collecting and storing actinospore stages is only a first phase of myxosporean research in the effort to reveal the infection of fishes and oligochaetes, the two alternative hosts of myxosporeans. Morphological characterisation of actinospore stages from oligochaetes and myxospore stages from fishes should be continued by molecular examinations in which 18S rDNA sequences of the morphologically defined myxosporean stages are compared. Completed sequences of both the actinospore- and myxospore stages in the future might help us to select identical spores of the same species, thus completing new life cycles of myxozoans from Lake Balaton. This early documentation of actinosporean stages from this biotope may encourage future research to improve our understanding on their life cycle and ecological structure among many important fish species of the Lake Balaton.

The number of oligochaetes studied for actinosporean infection in the Lake Balaton is rather high. Only in Scotland are more data available in this respect (Özer at al. (2002) (Table 4). Most of the studied species both in Hungary and other parts of the world belonging to *Tubifex tubifex* and *Limnodrilus* spp. In our case the majority of "large sized oligochaetes " belonged to *I. michaelsoni.* The controversy between our data and data in Table 4, can be explained by the changes in taxonomy of tubificid worms (personal communication by Timm, T. Estonia, who kindly identified our oligochaete specimens collected in this study). Besides data in Tables 1, 2, 3 a large number of "small sized" oligochaetes (*Nais*, *Dero, Aelosoma*) were examined, but evaluation of data received is in progress.

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