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**STUDIES ON FISH PARASITIC MYXOZOANS IN LAKE BALATON,
HUNGARY AND IN FRESHWATER AND MARINE BIOTOPES IN MALAYSIA**

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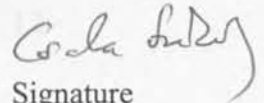
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ABSTRACT

Studies on Fish Parasitic Myxozoans in Lake Balaton, Hungary and in Freshwater and Marine Biotopes in Malaysia

There are only few data on the occurrence of parasitic myxozoans on Malaysian fishes. In this study, we report on collection of some new myxosporean species between 2010 and 2013 from this country. Four of them infected freshwater fishes; one was collected from an estuarine fish host. Besides morphological characterization of the species found, we made a molecular analysis on their 18S rDNA. In this study, we described *Myxobolus tambroides* sp. n. (60%) from the gills of an appreciated cyprinid, *Tor tambroides* collected from the Lake Kenyir Water-reservoir. Another new species elucidated was *Myxobolus ophiocarae* sp. n. (8.3% prevalence) found in *Ophiocara porocephala* (Gobiidae) collected from Merang Estuarine, Kuala Terengganu. The following specimens are yet to be described: *Myxobolus* sp. I (15% prevalence) was found in the muscle tissue of a *Labiobarbus* sp. (Cyprinidae). Sequences of *Myxobolus* sp. I resembled to *M. cyprini* with 94.6% similarity. *Myxidium* sp. I (66.7% prevalence) was found in the gall bladder of *Notopterus notopterus* (Notopteridae). *Myxidium* sp. II (15.4% prevalence) was found in the gall bladder of *Tor tambroides* (Cyprinidae). For the *Myxidium* sp. I, partial sequences of the specimen showed 90.6% similarity to *M. cuneiforme*; while complete sequences of *Myxidium* sp. II specimen collected from *T. tambroides* showed 87.2% identity to *M. anatidum*. Phylogenetic analyses of the 18S rDNA were performed to estimate and analyze relationships between myxozoan entities.

In another study made in Hungary after morphological analysis, we have examined 18S rDNA sequences of actinospores isolated from oligochaetes of Lake Balaton and Kis-Balaton Reservoir and compared them with sequences of known myxospore stages. In between 2010 to 2012, we studied the natural infection of the oligochaetes, *Branchiura sowerbyi* Beddard, 1892, *Isochaetides michaelsoni* Lastockin, 1936 and *Nais* sp. Müller, 1774. Thirteen actinosporean stages (4 triactinomyxon-types, 5 aurantiactinomyxon-types, 2 raabeia-types, 1 neoactinomyxon-type and 1 synactinomyxon-type) were found. Our molecular data showed that three types of actinospores (triactinomyxon-type 1, 2, 3) had 99.9-100% similarity to myxospores of *Myxobolus erythrophthalmi*, *M. shaharomae* and *M. fundamentalis*. Another complete sequence analysis of aurantiactinomyxon-type 4 collected from *B. sowerbyi* showed 99.4% match with *Thelohanellus kitauei* Egusa & Nakajima, 1981, though the myxospores of *T. kitauei* have not been recorded in Hungary hitherto. In addition, partial sequence of aurantiactinomyxon-type 5 isolated from a *Nais* sp., corresponded to *Thelohanellus nikolskii* Akhmerov, 1955 showing 99.9% similarity. Elucidation of the myxosporean fauna and their life cycle presented herein shows significant diversity among pre-investigated biotopes (Lake Kenyir, Merang Estuary, Lake Balaton and Kis Balaton). During this study, we have detected five new species of myxospores from Malaysia, we have presented new data on actinosporeans infecting oligochaetes in Lake Balaton and revealed the life cycle of five new myxosporeans in Hungary.

KIVONAT

Vizsgálatok Balatoni-, Valamint Malajziai Édesvízi- És Tengeri Biotópokon Élősködő Halparazita Nyálkaspóráson

Malajziai nyálkaspórák paraziták vonatkozásában napjainkig csak kevés szakirodalmi adat áll rendelkezésre. Disszertációmban a már meglévő ismeretanyaghoz további adatokat szolgáltatok, és beszámolok néhány 2010 és 2013 között gyűjtött új malajziai nyálkaspórák fajról, melyek közül négy faj édesvízi hal-gazdából, egy pedig brakkvízi halgazdából került kimutatásra. A kimutatott nyálkaspórák fajok morfológiai jellemzése mellett azok molekuláris biológiai módszerekkel való vizsgálatát (18S rDNS gén) is elvégeztem. Az első kimutatott fajt a Tasik Kenyir víztározóban élő, Malajziában magasan értékelt pontyféle, a *Tor tambroides* kopolyújáról gyűjtöttük, és azt *Myxobolus tambroides* néven új fajként írtuk le (Székely et al., 2012). A Kuala Terengganu közelében található Merang Lagunából származó *Ophiocara porocephala* nevű géb faj kopolyújáról ugyancsak kimutattunk egy új nyálkaspórást (8.3% prevalencia), melyet *Myxobolus ophiocarae* néven írtunk le. A Tasik Kenyir Víztározóból gyűjtött további fajok leírása folyamatban van. Ezek a következők: egy *Labiobarbus* sp. (Cyprinidae) izomzatában élősködő *Myxobolus* faj (15% prevalencia). Szekvenciája 94.6%-os genetikai hasonlóságot mutatott a génbankban megtalálható *M. cyprini* fajjal. *Notopterus notopterus* (Notopteridae) ephólyagjából izolált *Myxidium* faj (66.7% prevalencia). Részleges szekvenciája 90.6%-os hasonlóságot mutatott a *M. cuneiforme* fajjal, míg a *Tor tambroides* (Cyprinidae) ephólyagjából kimutatott *Myxidium* faj teljes szekvenciája 87.2%-os genetikai hasonlóságot mutatott a *M. anatum*-mal. A kimutatott új nyálkaspórák élősködők rendszertani besorolásához filogenetikai elemzéseket végeztem.

Magyarországi vizsgálataim során a Balatonból és Kis-Balatonból 2010 és 2012 között gyűjtött kevéssertéjű férgekből (*Branchiura sowerbyi* Beddard, 1892, *Isochaetides michaelsoni* Lastockin, 1936 és *Nais* sp. Müller, 1774) több aktinospora típust izoláltunk. Ezeket morfológiailag jellemeztük, majd molekuláris módszerekkel meghatároztuk 18S rDNS szekvenciájukat. A kapott adatokat egybevetettük a Génbankban megtalálható adatokkal. Összesen 13 aktinospora típust izoláltunk, melyekből 4 a triactinomyxon típusba, 5 az aurantiactinomyxon típusba, 2 a raabeia típusba, 1 pedig a neoactinomyxon típusba volt sorolható. Molekuláris vizsgálataink alapján az 1., 2. és 3. triactinomyxon típusok 99.9-100% hasonlóságot mutattak a *M. erythropthalmi*, *M. shaharomae* és *M. fundamentalis* nevű, Balatonból leírt nyálkaspórák fajokkal, melyek a vörösszárnyú keszeg, a kűsz és a bodorka parazitái. A *B. sowerbyi*-ből izolált 4. aurantiactinomyxon típus teljes szekvenciája 99.4% azonosságot mutatott a *Thelohanellus kitauei* Egusa & Nakajima, 1981 parazitával, jóllehet ezt a fajt még nem mutatták ki hazai halakból. Egy *Nais* fajból izolált 5. aurantiactinomyxon típus részleges szekvenciája 99.9% hasonlóságot mutatott a *Thelohanellus nikolskii* Akhmerov, 1955 fajjal. Az egyes biotópokon (Lake Kenyir, Merang Estuary, Balaton és Kis Balaton) vizsgált myxozoák előfordulása jelentős diverzitást mutatott. A munka során öt új malajziai nyálkaspórák fajt sikerült kimutatnunk, új adatokat szolgáltatnunk a Balatonban élő aktinosporákról, valamint tisztáztuk a Balatonban élő halakon élősködő öt nyálkaspórák faj fejlődési ciklusát.

ABSTRAK

Kajian ke atas Parasitik Myxozoa Ikan di Tasik Balaton, Hungary dan di Biotop Air Tawar dan Marin di Malaysia

Terdapat hanya beberapa data mengenai kehadiran parasit myxozoon pada ikan Malaysia. Dalam kajian ini, kami melaporkan koleksi baru spesies myxosporean antara tahun 2010 dan 2013. Empat spesies menjangkiti ikan air tawar; satu species dikumpulkan dari ekosistem muara. Selain pencirian morfologi spesies yang ditemui, analisis molekular 18S rDNA juga dijalankan pada mereka. Dari kajian ini, *Myxobolus tambroides* sp. n. dikenalpasti dari insang *Tor tambroides* yang diambil dari Tasik Kenyir (Székely et al. 2012). Satu lagi spesies baru dijelaskan adalah *Myxobolus ophiocarae* sp. n. (8.3% kekerapan) telah ditemui di *Ophiocara porocephala* (Gobiidae) diambil dari muara Merang, Kuala Terengganu. Spesimen berikut masih belum di identifikasi; *Myxobolus* sp. I (15% kekerapan) telah dijumpai dalam tisu otot *Labiobarbus* sp. (Cyprinidae). Jujukan sikuensi *Myxobolus* sp. I menyerupai *M. cyprini* dengan 94.6% persamaan. *Myxidium* sp. I (66.7% kekerapan) ditemui di dalam pundi hempedu *Notopterus notopterus* (Notopteridae). *Myxidium* sp. II (15.4% kekerapan) ditemui di dalam pundi hempedu *Tor tambroides* (Cyprinidae). Bagi *Myxidium* sp. I, sebahagian jujukan sikuensi spesimen menunjukkan 90.6% persamaan dengan *Myxidium cuneiforme*, manakala jujukan lengkap *Myxidium* sp. II yang dikutip dari *T. tambroides* menunjukkan identiti 87.2% kepada *M. anatidum*. Filogenetik analisis 18S rDNA telah dijalankan untuk menganggarkan dan menganalisis hubungan antara entiti myxozoon.

Selepas analisis morfologi, kajian jujukan 18S rDNA aktinospora yang diasingkan daripada oligocheta Tasik Balaton dan empangan Kis-Balaton dijalankan dan perbandingan mereka dengan jujukan peringkat myxospora dilakukan. Di antara tahun 2010 hingga 2012, kami mengkaji jangkitan semulajadi pada oligochaetes, *Branchiura sowerbyi* Beddard, 1892, *Isochaetides michaelsoni* Lastockin, 1936 dan *Nais* sp. Müller, 1774. Tiga belas aktinospora (4 triactinomyxon -jenis, 5 aurantiactinomyxon -jenis, 2 raabeia -1 neoactinomyxon -jenis dan 1 synactinomyxon -jenis) telah dijumpai. Data molekular menunjukkan tiga aktinospora (triactinomyxon - jenis 1, 2, 3) mempunyai 99,9-100 % persamaan dengan *M. erythrophthalmi*, *M. shaharomae* dan *M. fundamentalis* myxospores. Satu lagi analisis jujukan lengkap aurantiactinomyxon - jenis 4 menunjukkan 99.4 % persamaan dengan *Thelohanellus kitauei* Egusa & Nakajima, 1981, walaupun myxospores *T. kitauei* belum direkodkan di Hungary sehingga kini. Di samping itu, jujukan separa aurantiactinomyxon - jenis 5 dari *Nais* sp., sepadan dengan *Thelohanellus nikolskii* Akhmerov, 1955 dengan 99.9% persamaan. Myxosporeans fauna dan penjelasan kitaran hidup mereka yang dibentangkan di sini menunjukkan kepelbagaian yang ketara dalam biotopes yang disiasat (Tasik Kenyir, Muara Merang, Lake Balaton dan Kis Balaton). Kajian ini melampirkan lima spesies baru myxospores dari Malaysia, data baru aktinospora dan lima kitaran hidup baru myxozoa dari Hungary.