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Identification Species of *Myxobolus* from Gill of *Cyprinus carpio* in East Java

(IDENTIFIKASI MYXOBOLUS SP YANG DIPEROLEH DARI INSANG IKAN KARPER DI JAWA TIMUR)

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ABSTRACT

The aim of study was to identify *Myxobolus* sp. obtained from the gills of carp (*Cyprinus carpio*) of East Java, Indonesia. The cysts containing spores were collected from the gills of carp fish. The spores were examined by wet mounts preparation, fixed with ethanol absolute solution for molecular analysis. The spores had a transparent membrane, the shell, composed of two valves. The sutural ridge running between the valves. It was two anterior polar capsules, each consisted of a coiled polar filament. An iodinophilic vacuole and sporoplasm nuclei was located in posterior part. DNA Sequenses 18S rDNA followed by phylogenetic tree demonstrated that *Myxobolus* sp from Blitar was different from *Myxosoma cerebralis* of the Gene Bank. *Myxosoma cerebralis* was not found in the fresh water fish in Indonesia.

Keywords : Myxobolus sp, Cyprinus carpio.

ABSTRAK

Tujuan penelitian adalah untuk mengidentifikasi *Myxobolus* sp yang diperoleh dari insang ikan karper (*Cyprinus carpio*) di Jawa Timus, Indonesia. Kista yang berisi spora diperoleh dari ikan karper di Blitar (Jawa Timur). Spora diperiksa dengan metode *wet mount*, difiksasi dengan larutan etanol absolute untuk analisis molekuler. Spora memiliki *shell*, merupakan membran transparan yang terdiri atas dua valvula. Tonjolan berada di antara dua valvula. Spora memiliki dua polar kapsul anterior, masing masing terdiri atas kumparan filamen polar. Nukleus yang mengandung vakuola iodinofilik dan sporoplasma terletak di bagian posterior. Sekuen DNA pada 18S rDNA dari *Myxobolus* sp berasal dari Blitar berbeda dengan sekuen DNA dari *Myxosoma cerebralis* dari Gene Bank. *Myxosoma cerebralis* tidak ditemukan pada ikan air tawar di Indonesia.

Kata-kata kunci : *Myxobolus sp, Cyprinus carpio.*

INTRODUCTION

Indonesian government has embarked an intensification programme in aquaculture to be a greater fish producer in the world in 2015 (BKIPM, 2010). However, aquaculture is still obstacled by the problem of diseases and parasites, such as *Myxobolus* that caused high economical losses in the tropical countries (Kabata, 1985).

The first out break of *Cyprinus carpio* caused by *Myxobolus cerebralis pyriformis* infection was reported in Central Java, Indonesia, in 1951 by Sachlan (1952) and Djajadireja *et al.* ,(1982). High mortality of common carp (*C. carpio),* was caused by *Myxobolus* sp also occurred in Yogyakarta in 2002. (Kompas, 2002). Hobir (2006) found *Myxobolus cyprinii*, *Myxobolus koi,* and *Myxobolus artus* from gill of *C. carpio* from Magelang (Central Java) based on shape and size

of spores. Little is known about the occurrence of *Myxosporiasis* in Indonesia.

The genus of *Myxobolus* comprised several hundred species. Until now around 444 valid *Myxobolus* spp was reported from Eurasia and North America (Landsberg and Lom, 1991).
The spore shell of *Myxobolus* ovoid or pear shapped consisted of shell embarked along the line of suture lines. It has binucleate sporoplasms, embrionic nucleus, iodino philorus or glycogen vacuole, polar capsule, and polar filament (Lom and Dykova, 1992).

Morphological classification was often difficult to determine a species of *Myxobolus*. Molecular analysis approach could be used to support a traditional myxozoan classification that was often confusing (Eszterbauer *et al.,* 2001; Eszterbauer 2002).

The aim of research was to find out the species of Myxobolus and the presence of cerebralis from the fresh water fish in Indonesia.

RESEARCH METHODS

Myxozoans were collected from carpfarms, Blitar, East Java. Samples were fixed in 10 % formalin solution for histological examination. Some samples were fixed in ethanol for ribosomal DNA extraction using Qiagen Dneasy kit (Qiagen Inc, Hilden), then was amplified using polymerase chain reaction (PCR) with Myxo 18EF (5'- CTG GTT GAT CCT GCC AGT) and 18R (CTA CGG AAA CCT TGT TAC) primer pairs (Whipps *et al.*, 2003). Amplification products of rDNA were purified and sequenced to compare the isolate with sequences of *Myxosoma cerebralis* from Gene Bank.The sequence results were aligned with Mega program, and analysed with Maximum Parsimony method using 1000x bootstrap resampling (Sourdis and Neil, 1988).

RESULTS AND DISCUSSION

Cysts were found in the gills, brain, and the eye of common carp in Blitar. The spore shell of *Myxobolus* was ovoid or pear shapped consisted of shell adhering together along the lines of suture lines. It has binucleate sporoplasms, embrionic nucleus, iodinophilorus or glycogen vacuole, polar capsule, and polar filament (Fig. 1C).

The growth of cyst consisted of spores and infectious to other part of gills were very fast. The second lamellae of gill, was often gone and filled by cysts. The cysts were covered by a thick fibrous membrane (Fig.1B and 1D). The other part of gill were compensated by branchiectasis on the second lamellae (Fig. 1A) or sometimes hemorrhages.

Extraction and amplification products of gill, containing *Myxobolus* sp.from several areas in Indonesia (Muntilan, Blitar, and Bali) showed a good results (Figure 2).The length of band was about 1000bp. The results of ribosomal DNA sequences of *Myxobolus* sp. from Blitar were compared with *Myxosoma cerebralis* from Gene Bank. Their sequences were different from *Myxosoma cerebralis* (Figure 4).

The molecular study of *Myxobolus* have been done and showed 1600bp fragment of 18S rDNA region. The restriction fragment patterns of the PCR products generated by *Taq*I and *Msp*I enzymes to differ *M.elegans* from *M. hungaricus*. It could be seen that each pattern was characteristis and the pattern were easily distinguishable from each other (Eszterbauer, 2002).

The sequences of *Myxobolus* sp from Blitar, East Java (code number 5 and 6) were clearly differet from sequences of *M. cerebralis*. It proved that *M. cerebralis* have not been transmitted yet to Indonesian areas or supported the list of disease in Indonesia (BKIPM, 2010).

Whipps *et al.*, (2004) reported that *M. cerebralis* was close related to *Henneguya salmonicida*, using 28S primers. Andree *et al.*, (1999) have been reported about the relationship among member of the genus *Myxobolus*. However, two isolates of *Myxobolus* sp from Blitar Indonesia showed that they were very closed related to each other and one cluster with *M.cerebralis* using 18S primers (Figure 3 and 4).

CONCLUSIONS

The cysts of *Myxobolus* sp. from Blitar, East Java containing a lot of spores was covered by thick fibrous membrane. The cyst could grow fast, infectious, and metastasis as a tumour-like.

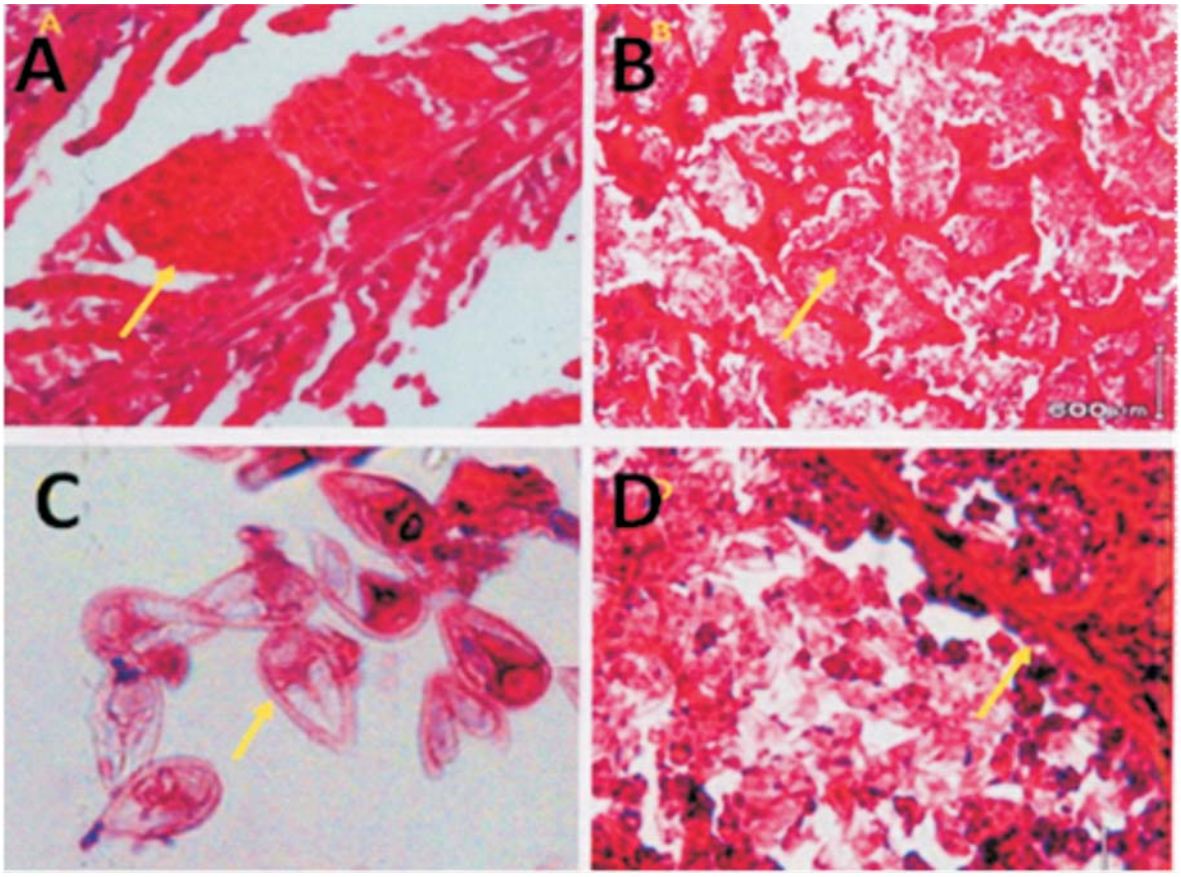

Figure 1. A. Branchiectasis on second lamellae of gill, B. Multiple cysts contained spores, *Myxobolus* sp., C. Individual spore *Myxobolus* sp. consisted of two polar capsules and iodinophilus vacuole posteriorly, D. Thick fibrous tissue of cyst membrane with many spores in the lumen. Scale bars of A,B,D 50µm; of C 20µm

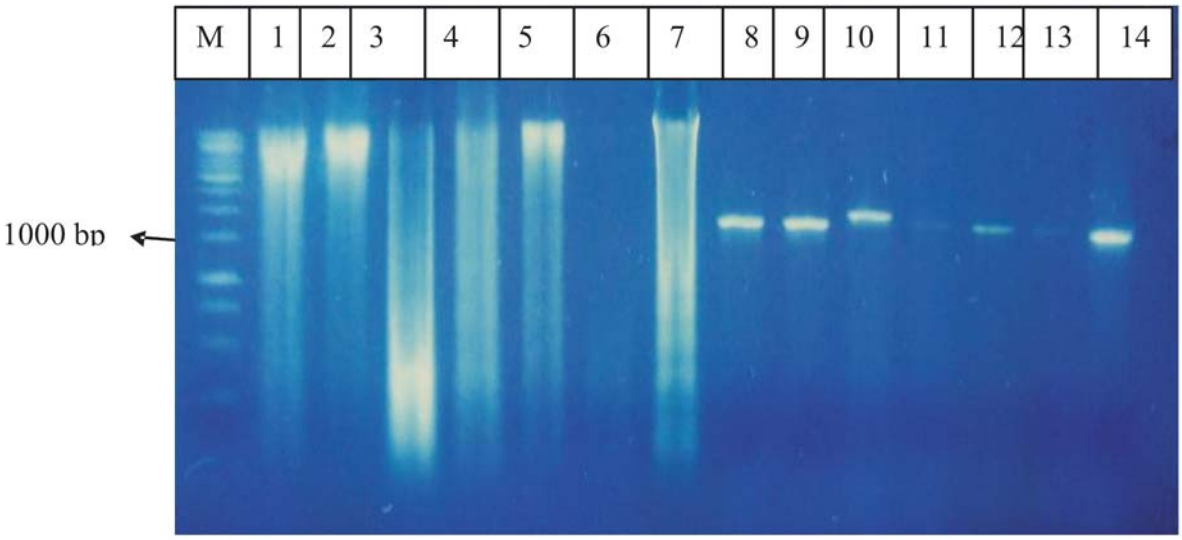

Figure 2. The extraction product of rDNA of *Myxobolus* sp. (lanes 1- 7) and the amplification results of *Myxobolus* sp. (Lanes 8 – 14). Amplification products of *Myxobolus* sp from Blitar were at lanes 8 and 9

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#Myxobolus_cerebralis_EF370480 CCTGCGGCTT AATTTGACTC AACACGGGAA AACTTACCAG GTCCG [ 45]
#Myxobolus A5                  ......... ......... .......... .C..C...C. .C...  [ 45]
#Myxobolus A6                  ......... ......... .......... .C..C...C. .C... [ 45]
#Myxobolus_koi                 ......... ......... ........G. ........T. ..... [ 45]

#Myxobolus_cerebralis_EF370480 GACATCAATA GGATAGACAG ACTGATAGAT CTTTCTTGAT ATGAT [ 90]
#Myxobolus A5                  ....CGG.A. ....T..... .T......C. ......C... TCTG. [ 90]
#Myxobolus A6                  ....CGG.A. ....T..... .T......C. ......C... TCTG. [ 90]
#Myxobolus_koi                 ......G.A. .......... .......... .......... GC.G. [ 90]

#Myxobolus_cerebralis_EF370480 GGATAGTGGT GCATGGCCGT TCTTAGTTCG TGGAGTGATC TGTCA [135]
#Myxobolus A5                  ..G.G..... .......... ........G. .....C...T ....T [135]
#Myxobolus A6                  ..G.G..... .......... ........G. .....C...T ....T [135]
#Myxobolus_koi                 .AG.G..... .......... .......... .......... ..... [135]

#Myxobolus_cerebralis_EF370480 GGCTAATCCC GGTAACGAAC GAGATCTTA- -TTCTCCATT TGATG [180]
#Myxobolus A5                  ..T.C..T.. .A........ ....CTCCGG C..GCTA.A. A.T.A [180]
#Myxobolus A6                  ..T.C..T.. .A........ ....CTCCGG C..GCTA.A. A.T.A [180]
#Myxobolus_koi                 ..T.T..T.. .......... ....C.ACC- -.......... .A.GA [180]

#Myxobolus_cerebralis_EF370480 AGCGGAAGAA GATAGTGTAG CTCGATGATT GTTTCGGCGA TTC-- [225]
#Myxobolus A5                  C....CCCC- .TGC.GTCG. .GTCC-A.C. TC..A.AG.G ACAAG [225]
#Myxobolus A6                  C....CCCC- .TGC.GTCG. .GTCC-A.C. TC..A.AG.G ACAAG [225]
#Myxobolus_koi                 .A.A.T.-GC AGG..GT.G. ..TA...-.. .C.....T.G C.--- [225]

#Myxobolus_cerebralis_EF370480 TCAAGTTATT CTATCGTAGG CAGTGTTTGT GAATTCAGCG TGAAA [270]
#Myxobolus A5                  .GGC...CAG .C.CGCG..A TG.A.CAATA AC.GGTCTGT GATGC [270]
#Myxobolus A6                  .GGC...CAG .C.CGCG..A TG.A.CAATA AC.GGTCTGT GATGC [270]
#Myxobolus_koi                 .TG.....GC ..-....... T..GA..GT. .....TTATA G.TGG [270]

#Myxobolus_cerebralis_EF370480 ATACAGTTTG TTGCGAGGAC GGGATAAAAC TCTTACTTGT TGCAA [315]
#Myxobolus A5                  CCT------- -------T.G AT.TCCGGGG CTGC..GC.C GC..C [315]
#Myxobolus A6                  CCT------- -------T.G AT.TCCGGGG CTGC..GC.C GC..C [315]
#Myxobolus_koi                 .--------- ------.TG. ....GGGC.A CTGA..G... .T..T [315]

#Myxobolus_cerebralis_EF370480 ATTGTACTAC ACCTGAGTTT GTTGGCATTC CCTTCCGTTA TACGC [360]
#Myxobolus A5                  .A..GG.GGA T.AGCGTG.G TC.---.CC. TGCG...AG. GG... [360]
#Myxobolus A6                  .A..GG.GGA T.AGCGTG.G TC.---.CC. TGCG...AG. GG... [360]
#Myxobolus_koi                 C.---GTG.A .G--...A.G A..----... ...A.T.... .G.AG [360]

#Myxobolus_cerebralis_EF370480 TGTTCAACTA CCCAGTTGAG CAGTGTGTCA TGGAGAGACT GTGAG [405]
#Myxobolus A5                  G.G.A.C.CG .TG.ACCCC. .-TC...ATC G...CT.GGG A.TGA [405]
#Myxobolus A6                  G.G.A.C.CG .TG.ACCCC. .-TC...ATC G...CT.GGG A.TGA [405]
#Myxobolus_koi                 ....GGGTA. AA.--C.TCA .GC..CC.T. .........A AC-.. [405]

#Myxobolus_cerebralis_EF370480 GTATATATCC AAGCTCAATG AAGCAAGGCC ATAACAGGTC TGTGA [450]
# Myxobolus A5                 AAC...T.-- CCCA....C. .G.A.TTC.. .GT.AGC.CG G..C. [450]
# Myxobolus A6                 AAC...T.-- CCCA....C. .G.A.TTC.. .GT.AGC.CG G..C. [450]
#Myxobolus_koi                 ..T....A-- ....CTG.G. ...TGT...T .......... A.... [450]

#Myxobolus_cerebralis_EF370480 TGCCCTA-AG ATGTCCTGGG CTGCACGCGC GCTACAATGA TGGTG [495]
#Myxobolus A5                  .AAG..CGC. T..ATTAA.T .CCTG.C.TT TG....-CAC C.CCC [495]
#Myxobolus A6                  .AAG..CGC. T..ATTAA.T .CCTG.C.TT TG....-CAC C.CCC [495]
#Myxobolus_koi                 ......T-C. ....T.A... .......... .......... .AACA [495]

#Myxobolus_cerebralis_EF370480 ACAGCGAGTT TCTAGGTCGA GAGACCTGGG CAATCTTGTA ATCAC [540]
#Myxobolus A5                  GTC..T.C.A -.CGAT.G.. TG.TTTA.T. AGG..C.CGG ...GG [540]
#Myxobolus A6                  GTC..T.C.A -.CGAT.G.. TG.TTTA.T. AGG..C.CGG ...GG [540]
#Myxobolus_koi                 ...A.A...A ...G...... A....T.... T.....-A.. ..TGT [540]

#Myxobolus_cerebralis_EF370480 CATCGTGATG GGGATTGACC ATTGTAATT- -TGGTCATGA AATAG [585]
#Myxobolus A5                  .CCGCC.GG. CTCC.C.CGG GCCC.GGCGG AGC.C.GA.. .GAC. [585]
#Myxobolus A6                  .CCGCC.GG. CTCC.C.CGG GCCC.GGCGG AGC.C.GA.. .GAC. [585]
#Myxobolus_koi                 T......... .........G .......A.- -.C....... ..... [585]

#Myxobolus_cerebralis_EF370480 GA [587]
#Myxobolus A5                  AT [587]
#Myxobolus A6                  AT [587]
#Myxobolus_koi                 .. [587]
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Figure 3. Sequence alignment of *Myxobolus* sp. from Blitar, East Java (sequences of number A5 and A6) were compared with sequences of *Myxosoma cerebralis ,Myxobolus koi,* and other species of *Myxobolus*.

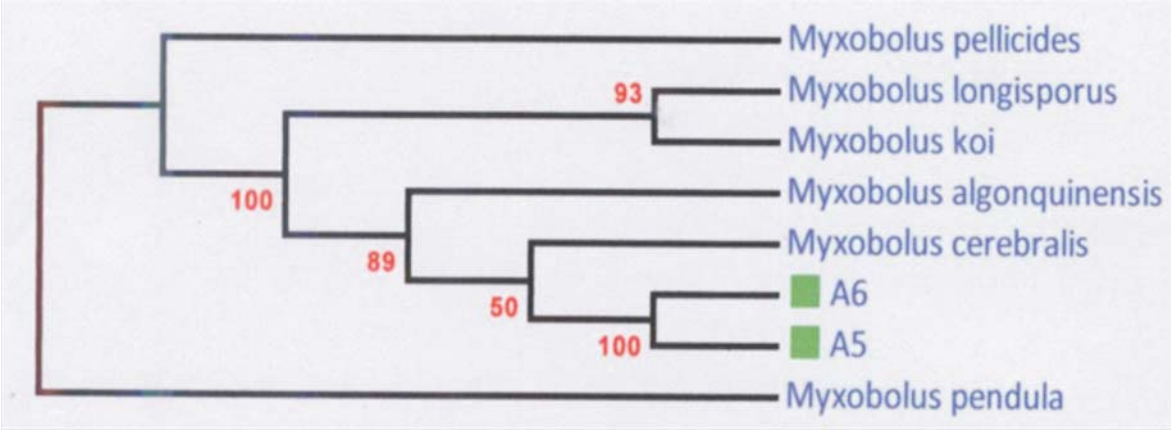

Figure 4. Filogenetic tree using maximum parsimony with 1000 bootstrap resampling of *Myxobolus sp*. isolat from Blitar compared to other *Myxobolus* from Gene Bank.

Myxobolus sp. from gill and brain of common carp in Blitar was different from *Myxosoma cerebralis* using 18S primers. *Myxosoma cerebralis* was not found from the fresh water fish in Indonesia.

SUGGESTION

It is recommended to have sequencing DNA using primer 18S rDNA from some samples from other islands of Indonesia for further detection of the gene rDNA. Moreover, it is also important to have further study using other method such as Restriction Fragment Length Polymorphism (RFLP).

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