

Intisari

Ikan gurami (*Osphronemus goramy* Lac.) adalah salah satu komoditas utama ikan indigenous Indonesia yang mudah terinfeksi saprolegniasis di setiap tahap pertumbuhannya. Penyakit ini beresiko pada kematian ikan, sehingga diperlukan kajian secara komprehensif. Penelitian ini bertujuan mengkaji saprolegniasis pada ikan gurami berdasarkan prevalensi saprolegniasis di lapangan, taksonomi dan filogeni jamur penyebab saprolegniasis, karakteristik infeksi dan respons ikan sebagai inang. Kejadian saprolegniasis dan miselium jamur dikoleksi dari ikan gurami budidaya di Jawa. Miselium diisolasi dan dikultur pada media *glucose yeast-extract*, air steril dan air steril-biji *Cannabis*. Karakterisasi dan identifikasi morfologi jamur berdasarkan struktur aseksual dan seksualnya. Identifikasi molekuler dan analisis filogenetik berdasarkan sekuen *Internal Transcribed Spacer Ribosomal Deoxyribonucleic Acid* (ITS rDNA) jamur yang disekuen dengan metode Sanger. Konstruksi pohon filogenetik dengan UPGMA, *neighbor-joining*, dan *maximum likelihood*. Karakteristik infeksi dan respons inang dianalisis berdasarkan gejala patologis, patogenisitas (persen infeksi dan *survival rate*), patologi integumen, hitung leukosit, dan protein mukus kulit ikan. Sediaan integumen ikan disiapkan dengan metode Bancroft dan jaringan darah dengan apus. Protein mukus dianalisis dengan SDS-PAGE dan *Liquid Chromatography High-Resolution Mass Spectrometry* (LC-HRMS).

Hasil pengukuran prevalensi saprolegniasis ikan gurami di pasar ikan 4%-50%, dan di kolam budidaya 0%-20%. Isolat jamur diidentifikasi sebagai *Newbya* (syn. *Achlya*) berdasarkan tipe pengeluaran spora *achlyoid*, dan oospora subsentrik atau sentrik. Seluruh isolat *monoecious*, hifa senositik, spora monomorfik, oogonium ber dinding halus, multioospora, membentuk *gemma*. Isolat kelompok-1 oosporanya subsentrik, anteridiumnya *diclinous* dan *diclinous-hypogynous*. Isolat kelompok-2 oosporanya subsentrik dan sentrik, anteridiumnya *diclinous*. Sekuen ITSrDNA isolat kelompok-1 tidak identik dengan *Achlya* lain yang memiliki nama penunjuk spesies (*identity* <94%), dan secara filogenetik merupakan *cluster* terpisah. Isolat kelompok-2 teridentifikasi sebagai *Newbya oblongata* (syn. *Achlya oblongata*) (*identity* 99,84%) mengelompok dengan *N. oblongata* LC149928.1. Uji patogenisitas menunjukkan 100% ikan gurami yang dipapar zoospora *Achlya* sp.5 terinfeksi. Infeksi ditandai tumbuhnya miselium *cottony* di permukaan integumen hingga lapisan dalam hipodermis disertai lesi kemerahan, nekrosis, ulserasi, kerusakan sirip di sekitar miselium, respons peradangan (berdasar gambaran patologi anatomi-histologis integumen dan apus darah), gangguan pergerakan dan keseimbangan ikan. Gangguan mukus kulit meningkatkan luasan infeksi dan menurunkan *survival rate* (SR 0% untuk infeksi dengan ami-momi dan 44,44% tanpa ami momi). Hasil SDS-PAGE menunjukkan deskripsi protein mukus kulit ikan terinfeksi dan non-infeksi serupa, tetapi protein 127kDa tidak terdeteksi pada mukus ikan terinfeksi. Uji LC-HRMS mukus kulit ikan gurami, mendeteksi protein terkait respons fisiologis (β -actin, *phosphotransferase*, *Cytochrome-C-Oxidase-subunit-2*, *GAPDH* (*phosphorylating*), *Sorting-nexin-33*, *Zic-Family-member1*, dan *V(D)J-recombination-activating protein-1*) serta protein terkait respons imun (*IL-1*, *TLR-3*, dan *TLR-22*).

Kesimpulannya, prevalensi saprolegniasis gurami di pasar ikan lebih tinggi dibandingkan kolam budidaya. Jamur yang menginfeksi teridentifikasi secara morfologi, molekuler, dan filogenetik sebagai *Newbya* sp (*Achlya* sp.) yang berpotensi sebagai spesies baru dan *N. oblongata* (syn *A. oblongata*). *Newbya* sp (*Achlya* sp.) patogen pada gurami, gejala



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Saprolegniasis pada Ikan Gurami (*Osphronemus goramy* Lacapède 1801) di Pulau Jawa //
Saprolegniasis in Gourami Fish (*Osphronemus goramy* Lacapède 1801) in Java Island

Siti Mukhlisah Setyawati, Promotor: Prof. Rina Sri Kasiandari, S.Si., M.Si. ; Co-promotor 1: Prof. Dr. Bambang Re

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patologis berupa tumbuhnya miselium *cottony* di permukaan integumen hingga hipodermis, nekrosis, ulserasi, kerusakan sirip disekitar miselium, respons peradangan, gangguan pergerakan dan keseimbangan ikan. Mukus kulit penting dalam perlindungan infeksi, respons fisiologis, dan imunologis ikan gurami sebagai inang.

Kata kunci: saprolegniasis, *Achlya*, *Newbya*, molekuler, filogeni, ikan gurami, patologi

Abstract

Gourami (*Osphronemus goramy* Lac.) was one of the main commodities of indigenous freshwater fish in Indonesia, which were easily infected by saprolegniasis at all stages of their life, so a comprehensive study is needed for infection control. This research aims to examine saprolegniasis in gourami based on the prevalence of the field's saprolegniasis, the taxonomy of saprolegniasis fungal agent based on morphological, molecular, and phylogenetic characteristics, characteristics of infection, and response of gourami as a host. The occurrence of saprolegniasis and fungal mycelium were collected from three main areas of gourami cultivation in Java. Mycelium was isolated and cultured on glucose yeast extract, sterile water, and sterile water-cannabis seeds. Characterization and identification of fungal morphology based on its asexual and sexual structures. Molecular identification and phylogenetic analysis based on sequences of *Internal Transcribed Spacer Ribosomal Deoxyribonucleic Acid* (ITS rDNA) of fungi sequenced by the Sanger method. Phylogenetic tree construction with neighbor-joining, and maximum likelihood. Characteristics of infection and host response were analyzed based on pathological symptoms, percent infection, survival rate, integument pathology, leukocyte count, and fish skin mucus protein. Fish were infected with zoospores through the immersion method. Fish integument preparation was prepared by Bancroft's method and blood tissue by smear. Mucus protein was analyzed in SDS-PAGE and *Liquid Chromatography High-Resolution Mass-Spectrometry* (LC-HRMS).

The results showed the prevalence of gourami's saprolegniasis 4%-50% in fish markets, and 0%-20% in cultivation ponds. Fungal isolates were identified as *Newbya* (syn. *Achlya*) based on the type of *achlyoid* spore release, and subcentric or centric oospores. All isolates were monoecious, coenocytic hyphae, monomorphic spores, smooth-walled oogonium, multi-oospores, and formed gemma. Group-1 isolates have subcentric oospores, *diclinous* and *diclinous-hypogynous* antheridia (this character has never been reported). Group-2 isolates have subcentric and centric oospores, and *diclinous* antheridia only. The ITS rDNA sequences of group-1 isolates were not identical to other *Achlya* with specific epithet names (identity <94%), and phylogenetically as a separated cluster. Group-2 isolates were identified as *Newbya oblongata* (syn. *Achlya oblongata*) (identity 99.84%) and clustered with *N. oblongata* LC149928.1. 100% of gourami fish exposed to *Achlya* sp.5 zoospores were infected on the second day. Infected fish characterized by the growth of cottony mycelium on the surface of the integument to the inner layer of the hypodermis accompanied by reddish lesions, necrosis, ulceration, damage to the fins around the mycelium, inflammatory response (based on the pathological anatomy-histology of the integument and blood smears), impaired movement and balance of the fish. Disruption of skin mucus increases the extent of infection and decreases the survival rate (SR 0% for infection with ami-momi and 44.44% without ami-momi). The SDS-PAGE results showed that the description of the skin mucus protein of infected and non-infected fish was similar, but a protein with 127kDa of molecular weight was not detected in the infected fish. LC-HRMS test of gourami skin mucus, detecting proteins related to physiological responses (*β-actin*, *phosphotransferase*, *Cytochrome-C-Oxidase-subunit-2*, *GAPDH* (*phosphorylating*), *Sorting-nexin-33*, *Zic-Family member1*, and *V(D)J-recombination-activating protein-1*) and proteins related to immune responses (*IL-1*, *TLR-3*, and *TLR-22*). In conclusion, the prevalence of gourami saprolegniasis in fish markets was higher than in aquaculture ponds. The infecting fungi were identified



morphologically, molecularly, and phylogenetically as *Newbya* sp (*Achlya* sp.), which can potentially be a new species, and *N. oblongata* (syn *A. oblongata*). *Newbya* sp (*Achlya* sp.) was a pathogen in gourami, pathological symptoms include the growth of cottony mycelium on the surface of the integument to the hypodermis, necrosis, ulceration, damage to the fins around the mycelium, inflammatory response, impaired movement and balance of the fish. Skin mucus is important in protecting against infection, physiological, and immunological responses of gourami as a host.

Keywords: saprolegniasis, *Achlya*, *Newbya*, molecular, phylogeny, gourami fish, pathology