ASSAYS ON THE AMPHIBIAN CHYTRID FUNGUS AT CROCKER RANGE, SABAH

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ABSTRACT

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Global amphibian population decline is happening at an alarming rate. Comparatively, anurans (Amphibia: Anura) face greater risk due to its greater representation in the Class Amphibia, and wider distribution throughout the Earth. *Batrachochytrium dendrobatidis* (Bd), the amphibian chytrid fungus, has been associated with the dramatic decline of amphibian and anuran populations globally. Thus, a study on the prevalence of the amphibian chytrid fungus on anuran population becomes imperative. The objectives of this study were (i) to investigate the prevalence of amphibian chytrid fungus, Bd, on anuran species at Crocker Range, Sabah and (ii) to assess the species composition of general fungal microflora on anuran species at Crocker Range. The study was conducted in Crocker Range which encompasses Crocker Range Park (CRP) and Kinabalu National Park (KNP). The altitude of the sampling sites ranged from 120 - 3,242 m a.s.l., temperatures ranged from 9 - 31^{o}C, and relative humidity ranged from 53 - 100%. Opportunistic examination was applied in anuran sampling. Swab-Polymerase Chain Reaction (PCR) approach was used to detect Bd. Swab-culture-based and PCR assay were used to isolate and identify the general fungal microflora from anuran skin. A total of 418 adult anurans representing 36 species, 17 genera and five families were collected. As for larval anurans, 298 larvae of *Hylarana* sp., *Leptobrachium* sp., *Meristogenys* sp., and *Rhacophorus* sp. were collected. A total of 507 specimens of anurans representing 418 wild adults, nine adult *Lithobates catesbianus*, 80 larvae with depigmented mouthpart, and three dead anurans were analysed for Bd presence. All collected anuran species were tested negative for Bd DNA. The reason for the absence of Bd in anurans at both Parks could be affected by temperature, altitude, latitude, and seasons, and varied response among anuran species towards Bd infection. Factors such as host age, innate defense such as the production of antimicrobial peptides, proportion of anti-chytrid microbes, and strains of Bd itself could also influence the prevalence of Bd. The lack of baseline information on the susceptibility of the sampled species to Bd infection is also preventing a definite conclusion regarding the absence of Bd in CRP and KNP to be drawn. A total of 19 genera of fungi representing 15 families and six classes from two phyla, namely Ascomycota and Basidiomycota, were identified from anuran skin. Further studies on the prevalence of Bd, and general fungal microflora on anuran species need to be conducted throughout Sabah, Borneo, and Malaysia.

Key words: *Batrachochytrium dendrobatidis*, anurans, swab PCR assay, Crocker Range, Sabah, Borneo

ABSTRAK

Kemerosotan populasi amfibia dunia sedang berlaku dengan kadar yang membimbangkan. Secara perbandingan, kumpulan anura (Amfibia: Anura) menghadapi risiko yang lebih serius disebabkan anura merupakan kumpulan yang terbesar di dalam Kelas Amfibia, dan mempunyai taburan yang luas di dunia. Batrachochytrium dendrobatidis *(Bd), kulat chytrid amfibia, telah dikaitkan dengan fenomena kemerosotan populasi amfibia dan anura di dunia. Oleh itu, kajian perlu dijalankan ke atas kadar sebaran kulat chytrid amfibia pada populasi anura. Objektif-objektif kajian ini adalah: (i) untuk menyiasat sebaran kulat chytrid amfibia, Bd ke atas spesies anura di Banjaran Crocker, Sabah dan (ii) untuk memeriksa komposisi spesies mikroflora kulat umum yang boleh didapati pada spesies anura di Banjaran Crocker, Sabah. Kajian ini telah dijalankan di Banjaran Crocker yang merangkumi kawasan Taman Banjaran Crocker (CRP) dan Taman Kinabalu (KNP). Kawasan persampelan berada dalam lingkungan ketinggian 120 – 3,242 m atas paras laut, suhu dari 9 - 31^{o}C, dan kelembapan relatif dari 53 - 100%. Kaedah 'opportunistic examination' diaplikasikan dalam persampelan anura. Kaedah 'swab-Polymerase Chain Reaction (PCR)' digunakan untuk mengesan kulat Bd. Kaedah swab-kultur-PCR digunakan untuk mengasing dan mengenalpasti mikroflora kulat umum daripada kulit anura. Sejumlah 418 anura dewasa mewakili 36 spesies, 17 genera and lima famili telah disampel. Untuk berudu, 298 berudu* Hylarana *sp.,* Leptobrachium *sp.,* Meristogenys *sp., dan* Rhacophorus *sp. telah disampel. Sejumlah 507 spesimen anura mewakili 418 anura dewasa liar, sembilan* Lithobates catesbianus *dewasa, 80 berudu dengan penyahpigmentasi bahagian mulut, tiga anura mati telah dianalisis untuk mengenalpasti kehadiran Bd. Semua spesimen anura didapati negatif untuk DNA Bd. Punca ketiadaan Bd pada anura di kedua-dua taman berkemungkinan dipengaruhi oleh suhu, ketinggian dan latitud kawasan, dan musim, dan reaksi berbeza anura terhadap jangkitan Bd. Faktor-faktor seperti usia perumah, sistem ketahanan imun dalaman seperti penghasilan peptida-peptida antimikrob, mikroorganisma anti-Bd, dan jenis Bd mempengaruhi kadar sebaran Bd. Kekurangan maklumat asas tentang kerentanan spesies anura yang disampel terhadap jangkitan Bd juga menyebabkan pengesahan ketiadaan Bd di dalam CRP dan KNP tidak dapat dimuktamadkan. Sejumlah 19 genera kulat mewakili 15 famili dan lima kelas dari dua filum iaitu Ascomycota dan Basidiomycota, diidentifikasi daripada kulit anura. Kajian-kajian lanjutan dan berterusan perlu dijalankan ke atas kadar sebaran Bd dalam populasi anura, dan komuniti mikroflora kulat umum dalam spesies anura di Sabah, Borneo dan Malaysia.*

Kata kunci: Batrachochytrium dendrobatidis*, anura, swab-kultur-PCR, Banjaran Crocker, Sabah, Borneo*