Abstract

Mangroves are important to the ecosystem as it protects the coastline from erosion, act as a nursery ground for the young sea life creatures and become a habitat for many organisms including hundreds of species of birds (both migratory and resident), monkeys, and lizards. Mangroves also provide a wide variety of substrates for fungi to grow. However, this study will focus on the lignocellulosic material only. Biodiversity of lignicolous mangrove fungi were sampled from three study sites of the Peninsular Malaysia mangrove forest. Cape Rachado (a sandy mangrove forest dominated by *Rhizophora apiculata*), Telok Pelandok (rocky shores with sandy mangroves dominated by *Avicennia* spp.) and Morib (a muddy mangrove forest dominated by *Rhizophora* spp.). Five hundred thirty six samples collected yielded 136 fungal taxa which comprises of 105 Ascomycota, 29 anamorphic fungi and 2 Basidiomycota. The most common (>10%) marine fungi from this study were *Dactylospora haliotrepha*, *Haloresellinia oceanica* and *Kallichroma tethys* which occurred in all 3 study sites. Species diversity was greatest in Telok Pelandok with 71 species recorded followed by Cape Rachado with 69. However, Cape Rachado has the highest Shannon-Weiner diversity index when evenness and total number of species occurrence were taken into account. Fungal species recorded showed little habitat recurrence as Sørensen similarity indices for all sites are below 0.5.

There are no phylogeography study done on marine fungi to date even though several terrestrial mycota have been examined with most of the studies on geographical differences of fungi are done on the terrestrial Basidiomycota. This is the first initiative study on investigating the phylogeography of marine fungi. *Verruculina enalia*, a marine mangrove fungus was chosen as the case study. The genetic relationship and variations of *V. enalia* from various biogeographical isolates was studied. The isolates of *V. enalia* are taken from Malaysia, Philippines, Hong Kong and Taiwan. One ITS

sequence of *V. enalia* isolated from Liberia deposited in GenBank was also included in the analysis. DNA sequence variation in nuclear ITS was examined to measure any genetic divergence among the isolates from different localities. Based on the phylogenetic analysis, isolates from Malaysia, Hong Kong, Philippines and Singapore are similar in terms of its sequences while isolates from Jici Rocky shore, Taiwan are different from the others to form a separate clade but differ for only less than 1%. *V. enalia* might possibly have not evolved long enough to accumulate mutations. Despite a strong bias toward sampling sites in the Southeast Asia region, this might be a signal that genetic variation among similar species that comes from the same ocean basin is minimal or does not have any variation at all which makes them totally identical. However, more isolates from wider geographical locations are required to confirm this.

Abstrak

Bakau penting kepada ekosistem kerana ia melindungi pantai daripada hakisan, bertindak sebagai pusat pembiakan dan pelindungan kepada hidupan laut yang kecil dan menjadi habitat bagi organisma yang lain seperti beratus-ratus spesis burung (pemastautin dan luar kawasan), monyet, dan biawak. Paya bakau juga menyediakan pelbagai substrat untuk pertumbuhan kulat. Walaubagaimanapun, fokus kajian ini akan hanya ditumpukan kepada material lignoselulosik sahaja. Biodiversiti kulat dari kayu bakau telah disampel daripada tiga kawasan kajian hutan bakau di Semenanjung Malaysia. Cape Rachado (hutan paya bakau berpasir yang didominasi tumbuhan *Rhizophora apiculata*), Telok Pelandok (pantai berbatu dan berpasir yang didominasi tumbuhan *Avicennia* spp.) dan Morib (hutan bakau yang berlumpur yang didominasi oleh *Rhizophora* spp.). Lima ratus tiga puluh enam sampel menghasilkan 136 taksa kulat yang merangkumi 105 Ascomycota, 29 kulat anamorfik dan 2 Basidiomycota, manakala yang selebihnya memerlukan pengecaman sepsis yang lebih lanjut. Kulat marin yang paling banyak direkodkan(> 10%) dalam kajian ini ialah *Dactylospora haliotrepha*, *Haloresellinia oceanica* dan *Kallichroma tethys* yang dapat ditemui di ketiga-tiga kawasan kajian. Kepelbagaian spesies di Telok Pelandok adalah yang tertinggi iaitu dengan 71 spesis diikuti oleh Cape Rachado dengan 69. Walau bagaimanapun, Cape Rachado mempunyai indeks diversity Shannon-Weiner yang tertinggi apabila keseragaman dan jumlah bilangan spesis yang dijumpai diambil kira. Spesis kulat yang direkodkan menunjukkan hanya sedikit sahaja yang berulang di kawasan kajian yang lain apabila kiraan persamaan Sorensen diantara kawasan kajian adalah di bawah 0.5.

Setakat ini masih belum ada kajian filogeografi yang dilakukan ke atas kulat marin walaupun beberapa kulat daratan telah dikaji dengan kebanyakan kajian perbezaan geografi kulat dilakukan ke atas Basidiomycota daratan. Ini merupakan

kajian inisiatif pertama untuk menyiasat filogeografi kulat marin. *Verruculina enalia*, sejenis kulat marin bakau telah dipilih sebagai subjek untuk kajian lanjut. Hubungan genetik dan variasi genetik *V. enalia* dari pelbagai lokaliti berbeza telah dikaji. Pencilan *V. enalia* diambil dari Malaysia, Filipina, Hong Kong dan Taiwan. Satu jujukan ITS *V. enalia* dari Liberia yang didepositkan di GenBank juga telah dimasukkan di dalam analisis ini. Variasi jujukan DNA dalam nuklear ITS telah diperiksa untuk mengukur sebarang penyimpangan genetik di antara pencilan dari lokaliti yang berbeza. Berdasarkan kepada analisis filogenetik, pencilan *V. enalia* dari Malaysia, Hong Kong, Filipina dan Singapura adalah sama dari segi jujukan manakala pencilan dari Pantai Berbatu Jici, Taiwan adalah berbeza dari yang lain untuk membentuk klad berasingan tetapi perbezaannya hanya kurang 1%. *V. enalia* mungkin tidak wujud begitu lama untuk mengalami mutasi. Walaupun persampelan dalam kajian ini berat sebelah dan hanya bertumpu di rantau Asia Tenggara, ini mungkin menjadi isyarat bahawa kepelbagaian genetik spesies yang sama yang berasal dari lembangan lautan yang sama adalah minimum atau tidak mempunyai lansung sebarang kepelbagaian dan ini menjadikan mereka betul-betul serupa. Walaubagaimanapun, lebih banyak pencilan dari lokasi geografi yang lebih luas diperlukan untuk mengesahkan kenyataan ini.

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Appendices
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General solutions
Culture media
Commercial Kits
Oligonucleotides primer
PCR Reagents
Agarose gel.

Appendix 2.0 Alignment of the ITS region for 16 taxa of lignicolous mangrove fungi using Clustal W (Higgins et al., 1994) in MEGA 4 (Tamura et al., 2007)

Appendix 3.0 Maximum parsimony tree (MP) of the ITS region

Appendix 4.0 Bootstrap maximum parsimony (MP) tree of the ITS region

Appendix 5.0 ITS region majority rule consensus tree of 60,001 post-burning trees sampled by the Bayesian search algorithm