

## ABSTRACT

Genus *Diadema* (Family Diadematidae) is among the most widely dispersed and ecologically vital sea urchins and they are the bioindicators in coral reefs and seagrass bed ecosystems. Although *Diadema* urchins had been widely studied, knowledge about their evolutionary process, population structure and intraspecific variation are less known. Phylogenetic and population structure analysis have been conducted using COI gene sequences to infer variation within and between population, and statistical analysis have been conducted to validate morphological differences within *D. setosum*. At the very beginning of this study, *Diadema* is hypothetically monophyletic based on COI gene analysis. *D. setosum* formed a monophyletic clade comprised of all individuals and further divided into two subclades, separating Red Sea population (*D. setosum*-b) from those of Indo west Pacific (*D. setosum*-a). *D. palmeri*, *D. clarki*, and the other five species (*D. mexicanum*, *D. antillarum*, *D. paucispinum*, *D. africanum* and *D. savignyi*) further formed their own subclades supported by high bootstrap value thus supporting interspecific variation. Population structure of *D. setosum* from Malaysian Borneo revealed two distinct geographical clades which separates Sabah populations (Clade II) from Sarawak populations (Clade I) following the theory of isolation by distance as genetic divergence values have direct correlation with geographical distances. Significant pairwise differentiation of estimated  $\Phi_{ST}$  value,  $D_a$  (COI = 1.85%) and lack of shared haplotypes between the two populations further suggest that historical events have had strong effects on the population structure of *D. setosum*. Findings suggests that Tanjung Datu undergo population reduction due to historical or recent bottleneck events based on the small and significant of the SSD and Harpending's raggedness, positive and lack of significance of the Tajima's D and the Fu's  $F_s$  value based on mitochondrial analysis. Findings suggests that historical events of

the West Baram Line, the Lupar Line, oceanic currents of the South China Sea, the SCS Southern Anticyclonic Gyre and transoceanic ship transport have strong effects on the population structure of *D. setosum* in Malaysian Borneo. In Satang Island, Sarawak, the existence of *D. setosum* morphotypes with white primary spines, green secondary spines and white peristomial membrane has raised concern over the issue on cryptic species complex and whether the island's ecological factors are the reason for the existence of *D. setosum* morphotypes. COI gene analysis suggest that white spines *D. setosum* samples found in Satang Island, Sarawak is a subspecies to *D. setosum* based on the formation of subclade and high genetic divergences between morphotypes, with significant differences in size, length-weight relationship, growth pattern as well as adaptability to environmental changes. Morphological data revealed the lack of significant differences between black spine and white spine *D. setosum* based on the twelve morphological characteristics however, subtle but identifiable distinctions of white primary spines, green secondary spines and white peristomial membranes were observed. The occurrences of *D. setosum* morphotypes in Satang Island, Sarawak might be due to the response of species to the environment in a specific scale as in Satang Island, Sarawak and the species may respond differently at larger scale which might be associated with habitat adaptation, natural selection or a physiological adaptation to ecological surrounding. Findings believes that cryptic species complex occurred in *D. setosum* with high morphological variability, due to fluctuating selection where high morphological polymorphism retained, causing the absence of some diagnostic characteristics of *D. setosum* such as the black spines and black peristomes. It is perceptible that COI gene is a suitable marker to infer genetic divergences at genus level, population structure and intraspecific variation.

**Keywords:** *Diadema*, *D. setosum*, population structure, morphotypes, COI gene

***Penstrukturan Filogenetik Landak Laut (Genus Diadema) Genetik Populasi Diadema setosum di Borneo Malaysia***

***ABSTRAK***

*Genus Diadema (Famili Diadematidae) ialah antara landak laut yang tersebar luas secara geografinya dan banyak ditemui di perairan cetek tropika, menjadi bioindikator bagi ekosistem terumbu karang dan hamparan rumpai laut. Walaupun kajian meluas berkaitan Diadema telah dijalankan, kefahaman berkaitan proses evolusi, struktur populasi dan variasi intra-spesifik masih terhad. Analisis filogenetik dan struktur genetik populasi D. setosum menggunakan gen COI dan analisis statistik untuk mengenalpasti perbezaan morfologi, telah dijalankan. Pada awal kajian ini, Diadema secara hipotesisnya adalah monofiletik berdasarkan analisis menggunakan gen COI. D. setosum membentuk kumpulan monofiletik yang kemudiannya terbahagi kepada dua kumpulan, mengasingkan D. setosum dari Laut Merah (D. setosum -b) daripada D. setosum dari Barat Indo Pasifik (D. setosum-a) dengan perbezaan genetik, perbezaan nukleotid, perbezaan subpopulasi yang tinggi, dan jumlah migrasi setiap generasi yang rendah. D. palmeri dan D. clarki, serta lima lagi spesies iaitu D. mexicanum, D. antillarum, D. paucispinum, D. africanum dan D. savignyi membentuk subkumpulan mengikut spesies yang menyokong variasi di antara spesies. Struktur populasi D. setosum dari Borneo, Malaysia menunjukkan terdapat dua kumpulan geografi yang berbeza iaitu populasi dari Sabah (Kumpulan II) dan Sarawak (Kumpulan I). Ini menyokong teori pengasingan oleh jarak (IBD) kerana variasi genetik berkadar terus dengan jarak geografi setiap populasi yang disokong oleh nilai perbezaan 'pairwise', Da (COI = 1.85%) serta kurangnya perkongsian haplotaip antara populasi tersebut. Populasi landak laut dari Tanjung Datu telah mengalami pengurangan nilai variasi genetik, berdasarkan data SSD dan Harpending, serta analisis positif Tajima dan Fu's Fs. Hasil kajian mencadangkan bahawa kewujudan Jurang Barat Baram, Jurang Lupar, arus Laut*

Cina Selatan serta kewujudan pusaran anti-siklonik 'SCS Southern Anticyclonic Gyre' serta peningkatan laluan kapal di Laut Cina Selatan adalah antara faktor yang mempengaruhi struktur genetik D. setosum di Borneo, Malaysia. Di Pulau Satang, Sarawak, kewujudan individu landak laut D. setosum yang mempunyai duri utama berwarna putih dan duri sekunder berwarna hijau serta membran putih di sekeliling peristome menimbulkan isi mengenai kewujudan spesies kriptik yang kompleks dan faktor ekologi Pulau Satang yang memainkan peranan dalam perbezaan morfologi akibat proses adaptasi. Analisis gen COI mencadangkan bahawa D. setosum berduri putih adalah sub-spesies, berdasarkan pembentukan sub kumpulan dan perbezaan genetik yang tinggi dengan D. setosum dan perbezaan signifikan dari aspek saiz, hubungan antara berat dan diameter, corak pertumbuhan serta adaptasi kepada perubahan alam sekitar. Data morfologi mendedahkan kurangnya perbezaan yang signifikan berdasarkan dua belas ciri morfologi yang dianalisis, namun begitu, perbezaan yang jelas terhadap kewujudan duri utama berwarna putih dan duri sekunder berwarna hijau serta membran putih di sekeliling peristome dapat diperhatikan. Kewujudan D. setosum yang mempunyai morfologi berbeza di Pulau Satang, Sarawak mungkin terjadi disebabkan oleh tindak balas spesies ini terhadap faktor ekologi dalam skala tertentu, seperti di Pulau Satang, Sarawak dan D. setosum mungkin bertindak balas secara berbeza pada skala yang lebih besar yang berkait rapat dengan adaptasi habitat, pemilihan semula jadi, atau peyesuaian fisiologi kepada persekitaran ekologi. Hasil kajian ini mencadangkan bahawa spesies kriptik wujud di dalam D. setosum yang mengawal polimorfisme yang tinggi dan menyebabkan ketiadaan beberapa ciri diagnostik spesies ini. Gen COI adalah penanda genetik yang baik bagi kajian variasi genetik pada tahap genus dan spesies serta struktur populasi.

**Kata kunci:** Diadema, D. setosum, struktur populasi, variasi morfologi, gen COI