

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Doctor of Philosophy

**PHENOTYPIC, PHYLOGENETIC AND SKIN TRANSCRIPTOMIC
ANALYSES OF DISCUS FISH (*Symphysodon* spp.)**

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ABSTRACT

Discus (*Symphysodon* spp.) is a popular ornamental fish species in the world, with Malaysia being one of the world's largest producers. With three distinct species native to the Amazon River in Brazil, discus have been successfully bred and raised in captivity, resulting in the production of many colour variations and varieties. In this thesis work, 69 discus varieties produced in Malaysia were categorized based on their primary (base) and secondary colours, as well as phenotypic characteristics. Given the significance of their genetic differences, genetic analyses of four wild discus varieties (Heckel, Brown, Blue, and Green) and ten selected domesticated discus (Blue Diamond, Ghost, Golden, Snow White, Albino Red Cover, Red Snakeskin, Golden Tiger, Rose Red, Pigeon Blood, and Leopard) were performed, revealing that the Green discus was the most distinct from the other wild discus, while Blue, Brown, and Heckel were closely related. Skin pigmentation cells were observed to better understand how colour and patterns were formed. RNA sequencing and bioinformatics technologies revealed genes and pathways involved in the production of colour. Melanin synthesis-related genes (ERBB4, ADCY9, WNT2, 14-3-3 proteins, and TSPAN genes) were upregulated in vertical bar regions, whereas carotenoids

metabolism-related genes (PLIN2, AHCY, RETSAT, STARD10, TTC39B, and RDH1 genes) were uniquely expressed in orange-spotted regions. A case study using red discus varieties indicated that the pteridine synthesis gene (*gch1* and *zgc:153031*), one-carbon metabolism (*aldh112* and *zgc:153031*), and lipid metabolism were regulated in the erythrophores and xanthophores of the Red Melon (RM) juvenile discus (*apoda* and *klf1*). The temporal inhibition of melanophore differentiation and development in RM juveniles might be attributed to *notum2* and *sost*, two genes implicated in Wnt-signalling inhibition. It is anticipated that as genetic engineering advances, the modification of pigment genes may facilitate new discus varieties with diverse body colours and patterns without the need for a time-consuming selective breeding process. Overall, this study provided insights on how discus varieties were categorized and distinguished, the latter crucial for standardization and identification, their genetic variation and pigment formation, all outcomes important for discus trade and other ornamental fish aquaculture.

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**ANALISIS FENOTIPIK, FILOGENETIK DAN TRANSKRIPTOM KULIT
IKAN DISCUS (*Symphysodon* spp.)**

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ABSTRAK

Ikan Discus (*Symphysodon spp.*) adalah spesies ikan hiasan yang popular dengan Malaysia sebagai salah satu pengeluar terbesar di dunia. Berasaskan tiga spesies yang berasal dari Sungai Amazon di Brazil, ikan Discus dengan pelbagai jenis variasi warna telah berjaya dibiakkan dan dibesarkan dalam kurungan. Dalam kajian tesis ini, sebanyak 69 varieti ikan Discus di Malaysia telah dikategorikan berdasarkan warna primer (asas) dan sekunder serta ciri-ciri fenotip. Memandangkan kepentingan perbezaan genetik ikan Discus, analisis genetik 4 ikan Discus liar (Heckel, Brown, Blue, dan Green) dan 10 varieti ikan Discus terpilih (Blue Diamond, Ghost, Golden, Snow White, Albino Red Cover, Red Snakeskin, Golden Tiger, Rose Red, Pigeon Blood, dan Leopard) telah dilakukan, dan hasil kajian menunjukkan bahawa ikan Discus Green adalah yang paling berbeza daripada ikan Discus liar yang lain, manakala ikan Discus Blue, Brown dan Heckel adalah berkait rapat antara satu sama lain. Analisis sel-sel pigmentasi kulit telah dilakukan untuk lebih memahami pembentukan warna dan corak ikan Discus. Teknologi penjujukan RNA dan bioinformatik telah digunakan untuk mengenal pasti gen dan tapak jalan yang terlibat dalam penghasilan warna. Terdapat peningkatan ekspresi gen berkaitan sintesis

melanin (gen ERBB4, ADCY9, WNT2, protein 14-3-3 dan TSPAN) di kawasan bar menegak, manakala gen berkaitan metabolisme karotenoid (gen PLIN2, AHCY, RETSAT, STARD10, TTC39B dan RDH1) diperhatikan secara unikny diekspreskan di kawasan berbintik oren. Kajian kes menggunakan varieti merah ikan Discus menunjukkan bahawa gen sintesis pteridine (gch1 dan zgc:153031), metabolisme satu karbon (aldh112 dan zgc:153031), dan metabolisme lipid (apoda dan klf1) kesemuanya mengawal eritrofor dan xantofor dalam ikan juvenil Red Melon (RM). Perencatan temporal pembezaan dan perkembangan melanofor ikan juvenil RM mungkin dikaitkan dengan peningkatan pengekspresan gen notum2 dan sost yang terlibat dalam perencatan isyarat Wnt. Adalah dijangkakan bahawa seiring dengan kemajuan kejuruteraan genetik, modifikasi gen pigmen memudahkan penghasilan varieti ikan Discus baharu dengan berbagai warna dan corak badan tanpa memerlukan proses pembiakan terpilih yang memakan masa. Secara keseluruhannya, kajian ini memberikan pemahaman tentang cara varieti ikan Discus dikategorikan dan dibezakan di mana adalah mustahak untuk pempiawaian dan pengenalpastian, perbezaan genetik dan pembentukan warna ikan Discus yang hasil-hasilnya adalah penting untuk perdagangan ikan Discus dan akuakultur ikan hiasan.