



UNIVERSITI PUTRA MALAYSIA

**PHYLOGENETIC RELATIONSHIPS AMONG MALAYSIAN
PUNTIUS AND ITS ALLIES (PISCES: CYPRINIDAE)**

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PHYLOGENETIC RELATIONSHIPS AMONG MALAYSIAN *PUNTIUS* AND ITS ALLIES (PISCES: CYPRINIDAE)

**By
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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment
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Chairman: Associate Professor Siti Khalijah Daud, PhD

Faculty: Science

The variation of criteria among authors and the discrepancies between morphological and molecular data generated a troublesome situation concerning the delimitation of the genus *Puntius*, which remains as yet unresolved. This is the first documentation on phylogenetic relationships among *Puntius* species in Malaysia integrating classical morphometrics, geometric morphometrics and molecular techniques. Morphological and phylogenetic studies were conducted on 10 Malaysian *Puntius* species and their allies, namely *P. bulu*, *P. fasciatus*, *P. schwanefeldii*, *P. binotatus*, *P. evereti*, *P. tetrazona partipentazona*, *P. tetrazona hexazona*, *P. daruphani*, *P. lateristeriga* and *P. gonionotus* obtained from five locations in Peninsular Malaysia.

A total of 312 samples were analysed. The quantitative data were analysed using analysis of variance (ANOVA), Principal Component Analysis (PCA) and

Discrimination Function Analysis (DFA). Discriminating efficiency was highest in the

truss network morphometric followed by classical morphometric and meristic techniques. In geometric morphometric Canonical Variate Analysis was performed on the total shape matrix. Results were further supported by sequencing of the cytochrome c oxidase subunit I (Cox1, 501 bp) and cytochrome b (Cytb, 857 bp) mitochondrial genes, and recombination activating gene (Rag2, 860 bp) and beta actin (β -actin, 911) nuclear genes. A combination of mitochondrial and nuclear data for phylogenetic relationships analysis was conducted from 39 representative samples of *Puntius* species. The phylogeny among taxa was constructed through Neighbor joining (NJ), Maximum parsimony (MP), Maximum likelihood methods (ML) and Bayesian inference (BI). The result of genetic distances and haplotype diversities among species were high indicating a complex genus of *Puntius*. In an attempt to confirm the phylogenetic position of *Puntius* within common cyprinids in Southeast Asia, dataset of *Puntius* in Peninsular Malaysia was complemented by Cytb and Rag2 sequences for different species of *Puntius* and common cyprinids belonging to Southeast Asia in GenBank. The tree with Maximum likelihood scores was chosen as the best tree. Clades retrieved from different trees strongly supported that subfamily Cyprininae and four selected genera within *Puntius* complex were monophyletic group, while tribe Barbinini, Cyprinini and genus *Puntius* were not monophyletic. Results of the combined data set were similar with the taxonomic hierarchy based on geometrical morphometric analyses. Divergence time estimated using a combination of molecular data from multiple DNA loci and fossil evidence for *Puntius* among Cyprininae was about 26.85 million years ago (26.08-28.22) and supported their early Oligocene origin.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

HUBUNGAN FILOGENETIK ANTARA *PUNTIUS* DI MALAYSIA DAN YANG BERKAITAN (PISCES: CYPRINIDAE)

Oleh

FAEZEH YAZDANI MOGHADDAM
Januari 2012

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Variasi kriteria di kalangan penulis dan percanggahan antara data morfologi dan molekul menghasilkan situasi yang menyukarkan persempadanan genus *Puntius* dan masih lagi belum dapat diselesaikan sehingga kini. Kajian ini merupakan dokumentasi pertama bagi perhubungan filogenetik di kalangan sepuluh spesies *Puntius* yang pertama di Malaysia yang mengintergrasikan teknik morfometrik konvensional, morfometrik geometrik and teknik molekular. Kajian morfologi dan filogenetik telah dijalankan ke atas 10 spesies *Puntius* Malaysia dan yang berkaitan, iaitu *P. bulu*, *P. fasciatus*, *P. schwanenfeldii*, *P. binotatus*, *P. evereti*, *P. tetrazona partipentazona*, *P. tetrazona hexazona*, *P. daruphani*, *P. lateristeriga* dan *P. gonionotus* yang di perolehi dari lima lokasi di Semenanjung Malaysia.

Sejumlah 312 sampel telah dianalisis. Kuantitatif data dianalisis menggunakan analisis varians (ANOVA), Analisis Komponen Utama (PCA) dan Analisis Diskriminasi (DFA).

Kecekapan diskriminasi adalah paling tinggi pada teknik morfometrik Truss. diikuti dengan morfometrik biasa dan meristik. Bagi morfometrik geometri, analisis varians kanonikal di jalankan ke atas matriks bentuk total. Keputusan telah di sokong oleh jujukan gen mitokondrial DNA pada sitokrom c oksida subunit I (CoxI, 501bp) dan sitokrom b (Cyt b, 857 bp), serta gen nukleus iaitu gen rekombinasi teraktif 2 (Rag2, 860bp) dan beta aktin (β -actin, 911 bp). Kombinasi data mitokondria dan nukleus dilakukan ke atas 40 sampel spesies *Puntius*.

Filogeni antara taksa dibina dengan menggunakan 'Neighbour Joining' (NJ), dan 'parsimoni maksimum' (MP), kaedah 'Maximum likelihood' (ML), dan inferensi Bayesian (BI). Jarak genetik dan diversiti haplotip antara spesies adalah tinggi, menunjukkan *Puntius* adalah genus yang kompleks. Cubaan untuk mengesahkan posisi filogenetik *Puntius* dalam siprinid di Asia Tenggara, data *Puntius* dari Semenanjung Malaysia telah dilengkapkan dengan jujukan Cytb dan Rag2 bagi spesies *Puntius* yang berlainan dan siprinid bias ayang berasal dari Asia Tenggara yang terdapat di Genbank. Pohon dengan skor Parsimoni maksimum dipilih sebagai pohon terbaik. Klad yang diperolehi daripada pohon yang berbeza menyokong kuat kewujudan subfamili Cyprininae dan empat genus dalam kompleks *Puntius* yang terpilih adalah kumpulan monofiletik., manakala suku Barbinini, Cyprinini dan genus *Puntius* adalah bukan monofiletik. Keputusan daripada kombinasi set data menunjukkan persamaan dengan hirarki taksonomi berasaskan analisis morfometri geometrik. Masa pencapahan yang dianggarkan dengan menggunakan gabungan data molekul daripada lokus DNA berganda dan bukti fosil DNA *Puntius* di antara Cyprininae adalah lebih kurang 26.85 juta tahun yang lalu (26.08-28.22) dan ini menyokong ia berasal daripada zaman Oligocene awal.

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I certify that a Thesis Examination Committee has met on 20/Jan/2011 to conduct the final examination of FAEZEH YAZDANI MOGHADDAM on her Doctor of Philosophy thesis entitled “The Systematics of Malaysian *Puntius* and Its Allies (Pisces: Cyprinidae) ” in accordance with the Universities Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Doctor of Philosophy. Members of the Thesis Examination Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

FAEZEH YAZDANI MOGHADDAM

Date: 20 January 2012



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