

Phylogenetic Relationships Within the Labeoin Group Inferred from Mitochondrial 16S rRNA Sequences and Morphometric Analysis

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The Labeoin is a group of freshwater fishes from the family Cyprinidae commonly found in Malaysia and throughout Southeast Asian region. The taxonomic status of many Labeoin group has been well-defined [1,4]. However, there still remains some controversy over several presumed species. We summarize here the results obtained based on the investigation of the mitochondrial (mtDNA) 16S rRNA gene on four species and morphometric truss network on five species within this group. Although these species are clearly characterized by conventional morphological method, this study offers alternative tools to investigate other less well defined species within this group. This paper forms part of our on-going multi approach investigation on the Labeoin group in Malaysia and Cambodia.

Sample collection

The samples as summarized in Table 1 were either caught or purchased from fishermen.

Table 1 Sample profiles used in analysis

Species	Sites	N (rRNA)	N (morphometric)
<i>L. festivus</i>	Tasek Bera	5	34
<i>L. leptochelilus</i>	Tasek Chini	5	20
<i>M. chrysophekadion</i>	Tonle Sap Lake	*	20
<i>O. hasselti</i>	Tasek Chini	5	29
<i>O. vittatus</i>	Ahning Dam	4	4
	Tasek Bera	1	1

* Extraction was unsuccessful

Molecular studies

Fish fin/ muscle tissues were immediately preserved in TNES-Urea buffer upon sampling. DNA was extracted by PURE GENE Tissue DNA Kit. The extract was PCR-amplified for a partial segment of the 16S rRNA mtDNA gene using the primer pair L1567 and H2196 (M. Masaki- unpublished). The PCR protocol was according to Norfatimah [2]. The PCR product was then electrophoresed and visualized by staining with ethidium bromide. Purification of PCR product was carried out using QIAquick PCR Purification Kit followed by sequencing. The sequences obtained were aligned using the software Clustal X followed by phylogenetic analysis using PAUP software [5].

Morphometric studies

Twenty six truss measurements (and standard length) were measured using a pair of digital calipers. In order to standardise the different sizes of specimen due to differing allometric growth, a transformation formula [3] was applied to the raw data. A multivariate analysis, Discriminant Function Analysis (DFA) was then performed using SPSS software version 11.5.

Mitochondrial DNA analysis

A partial segment of about 730 bp of the 16SrRNA mtDNA gene was successfully amplified. The dendrogram (Fig. 1) showed that all samples were correctly assigned to their respective species. However, individual *O. vittatus* 5 (from Tasek Bera), formed an outgroup for the *O. vittatus* and *O. hasselti* clusters.

Morphometric analysis

Results of ANOVA showed that all characters were significant. Based on the standardised canonical discriminant function coefficient the head region, diagonal depth of caudal peduncle and mid-body depth were identified as the most important variables in discriminating the four Labeoin species. The multivariate cluster analysis of the morphometric data also showed similar results with complete differentiation among the five species (Fig. 2) based on their initial identification by traditional characters.

In the molecular analysis almost all specimens were classified according to their species except a single specimen of *O. vittatus* which was isolated from its own cluster suggesting misidentification or presence of a new taxon. The morphological characteristic of *O. vittatus* 5 was found to show some differences; appearance of red spots on the abdominal area as in *O. hasselti* suggesting that this specimen is closely related to *O. hasselti*. Thus, the taxonomic status of this problematical specimen *O. vittatus* 5 need to be further clarified. This study shows the potential of 16S rRNA mtDNA in species discrimination within the Labeoin group. The results obtained was also supported by the truss network morphometric technique. Further analysis is being conducted on nine other species including three from the Tonle Sap Lake in Cambodia.

Figure 1 Dendrogram generated by sequencing of partial fragment of 16S rRNA mtDNA gene of *L.festivus* (*L.fstv*), *L.leptocheilus* (*L.lpt*), *O.hasselti* (*O.hslf*) and *O.vittatus* (*O.vit*).

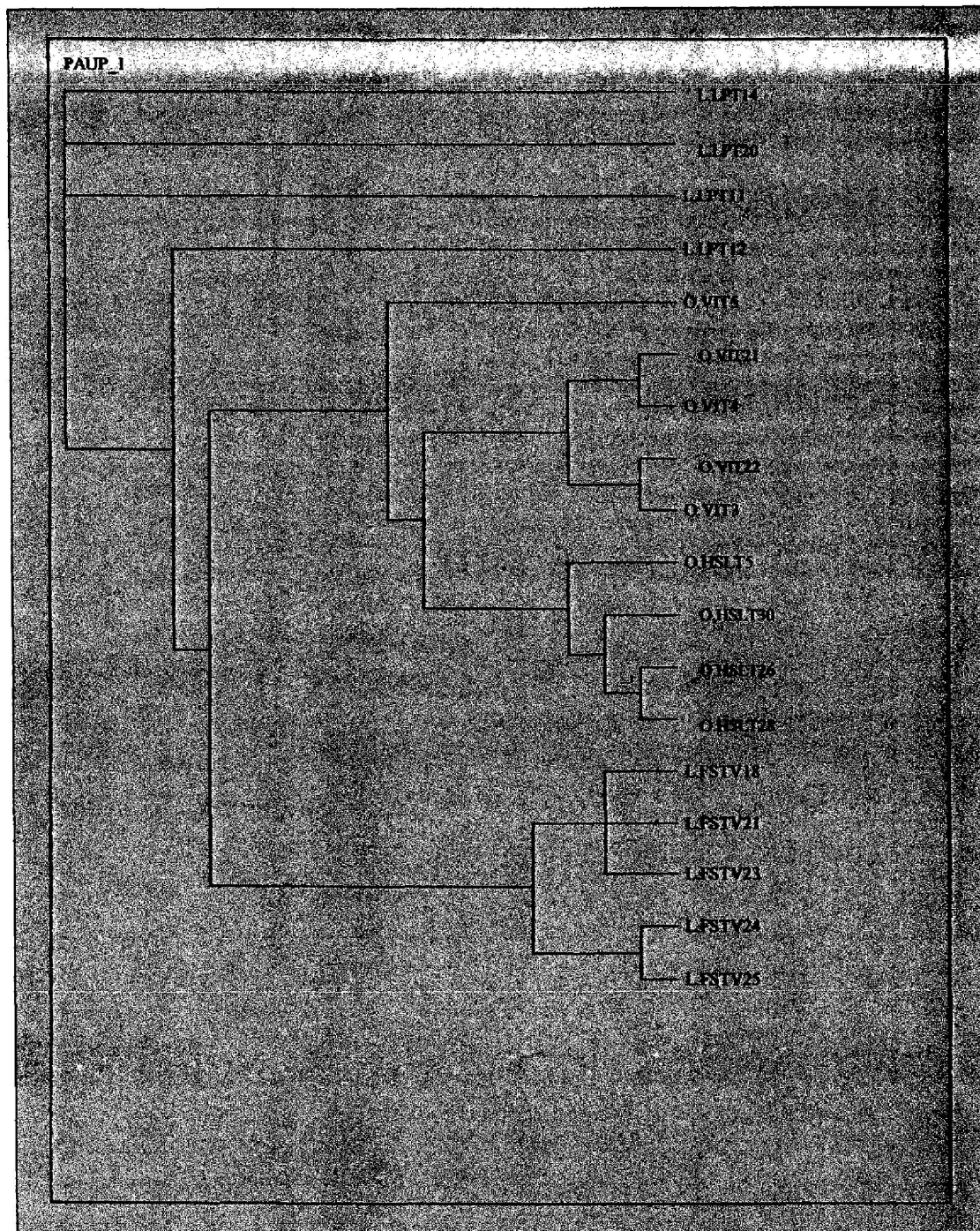
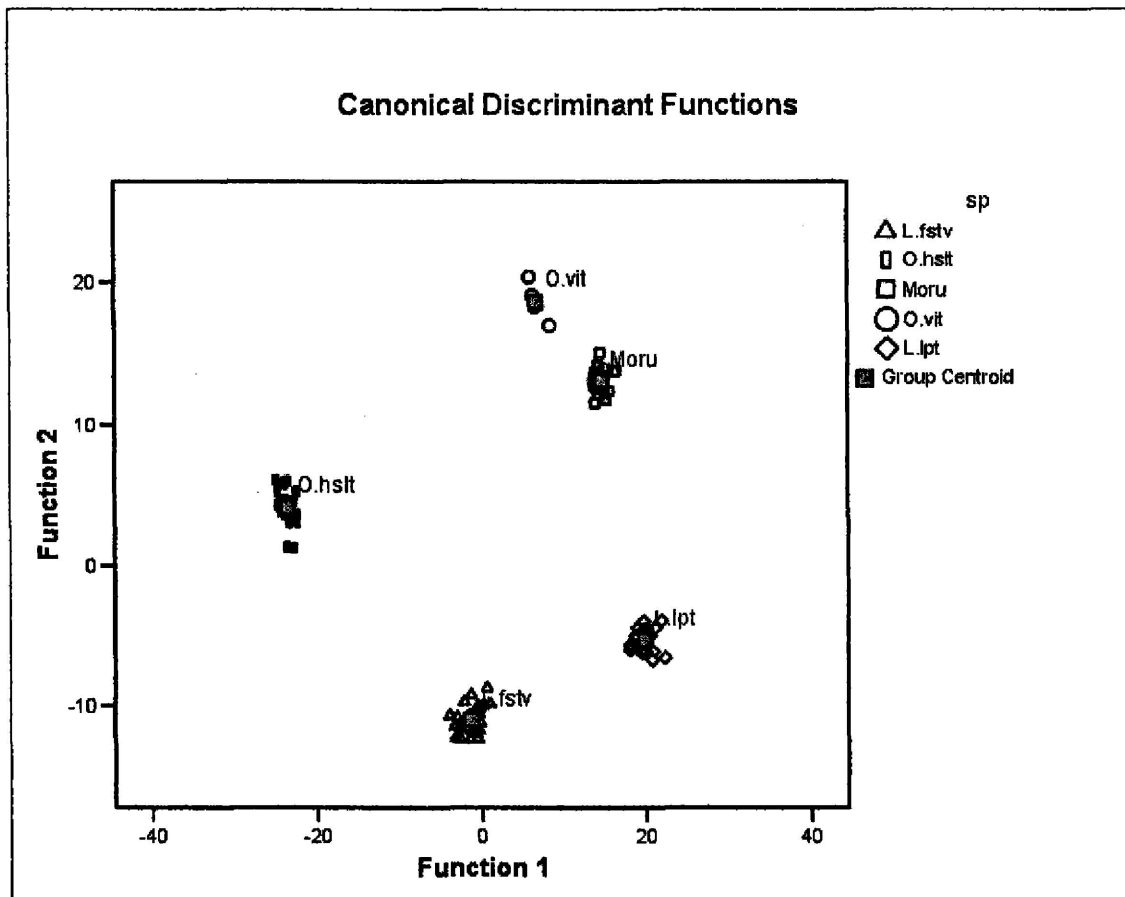


Figure 2 Scatter plot generated of Function 2 vs. Function 1 in the DFA analysis



The finding shows the potential of both 16S rRNA mtDNA gene and morphometric characters in the systematic study of the Labeoin group. It is hoped that this study will contribute in clarifying the taxonomic status of this group of fishes.

References

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