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Phylogenetic Analysis of Crayfish Species Using Mitochondrial DNA Markers

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Abstract

Phylogenetic analysis of crayfish species was executed using sequences from three mitochondrial gene regions: 16S rRNA, cytochrome oxidase I (COI), and cytochrome b (Cyt b). This analysis is crucial for molecular taxonomic identification and biodiversity assessment because it determines the level of variation in gene regions. However, information data on potentially mitochondrial gene regions are lacking for phylogenetic studies in commercial crayfish species. The three regions of mitochondrial genes were used in this study to determine phylogenetic relationships between crayfish species. The phylogenetic trees were constructed to better understand the evolutionary relationship between crayfish species. The neighbour-joining (NJ) and maximum parsimony (MP) methods were used to evaluate the 16S rRNA, Cyt b, and COI genes, which act as molecular markers for the genus *Cherax* and are used to infer phylogenetic relationships. The composition of nucleotides and their properties were compared among three regions of mitochondrial genes. Phylogenetic analyses between *Cherax* species revealed that 16S rRNA was the least variable, with a range of 3.8-17.38%, and COI was the most variable, with a range of 4.9-25.74%. All *Cherax* species are consistently grouped together using three regions of mitochondrial genes. The COI gene sequences were more conserved than the Cyt b and 16S rRNA gene sequences. All of the gene sequences provided sufficient phylogenetic information to distinguish between crayfish species, and thus could be useful molecular markers for species identification.

Keyword: Crayfish; phylogenetic; molecular marker

INTRODUCTION

Crayfish have become important in the aquaculture, pet trade, and ornamental aquaculture industries, with several species being more or less exploited. Despite the fact that crayfish are a diverse taxonomic group, some species are critically endangered and others are highly successful invasive species [1], [2]. Furthermore, invasive species can be detrimental to a species' survival, particularly if they are followed by hybridization and introgression. Because uncommon species are more prone to genetic swamping, the risk of introgression becomes

more apparent when they come into contact with more common species. If the introgression spreads across the species, the population may become extinct, and recovery will be impossible. Furthermore, mitochondrial genes are distinct from one another, particularly in terms of evolutionary rate variability at different loci [3]. Due to these conditions and a lack of work done to establish the identities and phylogenetic relationships of commercial crayfish, mitochondrial DNA is being used to study the molecular relationship among selected crayfish species. Mitochondrial DNA markers, 16S rRNA, Cyt b, and COI were compared to evaluate which one is more conserved and evolves at a slower rate.

METHODOLOGY

The DNA sequence data from 13 crayfish species of the three region locus (COI, Cyt b, and 16S rRNA) of mtDNA sequences were used in this study. The nucleotide sequences were obtained from the NCBI website: https://www.ncbi.nlm.nih.gov/. The sequences were then aligned on MEGA X using the MUSCLE Alignment tool. The MEGA X software was then used to generate a phylogenetic tree using the MP criteria. A NJ analysis based on the p-distance parameter (distance measurement) was also performed using MEGA X to evaluate genetic distance between crayfish species and aid in evaluating topology achieved by parsimony tree. Bootstrap analysis with 1000 replicates was used to assess relative support at nodes.

RESULTS AND DISCUSSION

Table 1 displays the percentage average of the base composition of each gene as the character status of the MP analysis from the loci of 16S rRNA, Cyt b, and COI. The base composition varies slightly between the compared regions. In this study, the base composition of 16S rRNA was identified to be less conserved (23.23%) and less variable (41.67%) when compared to the Cyt b and COI gene regions. The COI gene was the most conserved, but the region's base composition was highly variable when compared to 16S rRNA. Similar to *Cherax destructor*, the degree of divergence for the 16S rRNA gene regions was found to be the lowest when compared to COI, COIII, and adenosine triphosphatase 6 (ATPase 6) [4]. Furthermore, the overall levels of divergence are consistent with previous findings for a wide variety of organisms [5]. At the interspecific level, the average divergence within *Cherax* species is comparable to that demonstrated in other freshwater crayfish genera that use this gene region.

Table 1 Character status (%) of maximum parsimony from three mitochondrial gene regions 16S rRNA, Cyt b and COI gene markers

Characters	16S rRNA	Cyt b	COI
Conserved (constant)	23.23%	27.37%	34.35%
Variable (uninformative)	41.67%	58%	58.97%
Parsimony informative	25.94%	24.02%	35.88%

The overall result revealed that the parsimony tree was composed of sub-cluster A (*C. quadricarinatus*, *C. boesemani*, *C. bicarinatus*, *C. holthuisi*, *C. glaber*, *C. destructor*, *C. crassimanus*, *C. preissii*, *Procambarus clarkii*, *Macrobrachium rosenbergii*, *C. monticola*, *Homarus americanus* and *C. cainii*), sub-cluster B (*C. rotundus*, *C. tenuimanus* and *Engaeus*

strictifrons), sub-cluster C (*C. quinquecarinatus*, *Ictalurus punctatus* and *Paracheirodon axelrodi*) and sub-cluster D (*Panaeus monodon* and *Panaeus indicus*). The first and second clades are dominated by *Cherax* species, while the third clade contains only one *Cherax* species and the fourth clade is dominated by *Panaeus* species (sub-cluster D). The phylogenetic analysis revealed that the *Cherax* species were grouped together in a monoplyletic group. The group was supported by a large number of bootstraps in the NJ tree (93%) (figure not shown) and the MP tree (78%) (Figure 1). In this tree, *Penaeus* species were placed in a different clade than *Cherax* species. The *Penaeus* species were regarded as outgroup species. This finding was supported by a previous study conducted by Munasinghe et al. [5] on the molecular phylogeny of the freshwater *Cherax erichson* using 12S rRNA and 16S rRNA genes. The *Cherax* species was considered monophyletic, and the results of this study are consistent with what they have reported [4–6]. Variability differences in a specific region of mtDNA could be caused by a variety of factors, including the evolutionary rate between loci, sample sampling, and differences in the rate of sequence divergence between genera [5].

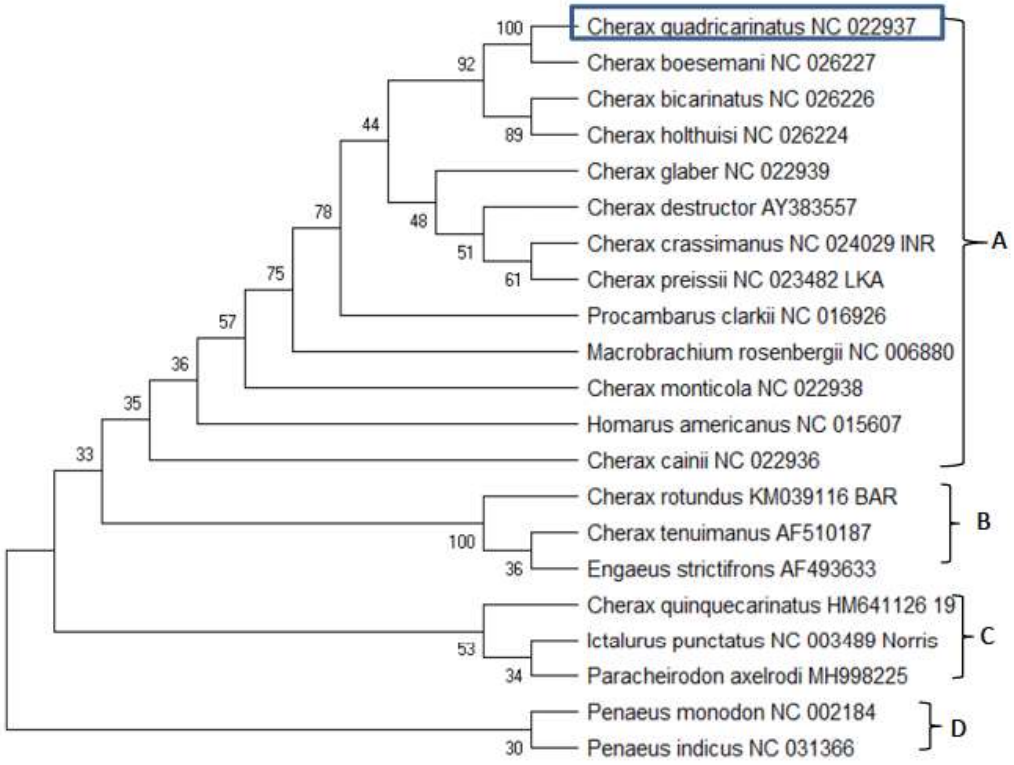

Figure 1 Maximum Parsimony tree for *Cherax* species using mitochondrial genes

CONCLUSION

This study contributed information about the phylogenetic relationships among *Cherax* species by using 16S rRNA, Cyt b, and COI sequences. The phylogenetic tree obtained in this study suggests that *Cherax* species are monophyletic in the crayfish. This study provided additional support for the phylogenetic relationships among crayfish species presented in previous studies, though the current findings still require clarification through the collection of indigenous crayfish, particularly using nuclear data.

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