Molecular Systematics of Some Shrimps Based on Allozyme Data
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Abstract: Previously proposed phylogenetic hypotheses for the family Penaeidae based on morphological characters receive little. Molecular data, which are less affected by environmental factors and can distinguish homology from analogy, are an additional data set in phylogenetic reconstruction. Although morphological and molecular characters evolve independently. Molecular phylogenetic studies have been conducted encompassing all the common and a few rare penaeid genera aiming at elucidating phylogenetic and evolutionary relationships at the generic (15 genera) and family level. Of 14 loci scored, the phylogenetic relationships by cluster analysis largely agree with the phylogenetic hypothesis of 3 tribes. The commonly adopted evolutionary polarity of the family from *Penaeus* to *Trachypenaeus* was also confirmed.

Key words: Allozyme, shrimps, Penaeus, Metapenaeus, Parapeneaepsis, Trachypenaeus

INTRODUCTION

Molecular assays have opened the entire biological world for genetic scrutiny; provide common yardsticks for measuring divergence of species[32]. Different types of molecular assays[20] provide genetic information ideally suited to different subsets of this hierarchy, and a continuing challenge to develop and utilize molecular methods appropriate for a particular biological problem at hand.

Penaeid shrimp is one of the most important crustaceans due to their importance in ecology and economy[25,29,52,40], and in phylogenetic studies of higher taxonomy of crustaceans[8,5]. Despite their distribution over different oceans of the world, penaeids are predominately as Indo-West Pacific fauna. A total of 17 genera and more than 190 species of Penaeidae were recorded[17] and the family has been subjected to intensive taxonomic revisions[25,29,40,12,13,14,15]. Phylogenetic relationships among genera of Penaeidae have been studied based on comparative or functional morphology, and different grouping schemes have been proposed. For example, Burkenroad[9] proposed a phylogenetic grouping for by dividing 15 penaeid genera into 3 tribes. Kubo[27] proposed a phylogenetic consideration by separating 9 genera of penaeids from the Indo-West Pacific into 5 groups. Because different characters are emphasized in two studies, these morphological phylogenies differ not only in the branch order but also the evolutionary polarity. Limited fossil records and incomplete palaeogeographic evidence are of little significant in phylogenetic studies of penaeids[17,40]. Molecular data thus appear to be the only available approach to challenge the previous taxonomic revision and phylogenetic groupings[17].

Molecular approaches have been useful in generating data for different phylogenetic analyses[2,8,19,49,48,24,30,25,45,31,26]. Allozymes as a genetic marker have found widespread use in population genetics[2,8,38], and phylogenetic analysis in different groups of decapods such as crayfish[19,11,16] and lobsters[10]. Previous molecular phylogeny on penaeids is scarce and restricted to only two genera, *Penaeus* and *Metapenaeus*[15,46,11]. The results of these studies, together with other evidence, if any, have supported or rejected previous phylogenetic hypotheses. No molecular data are available in other genera of Penaeidae. In the present study, allozyme analysis was conducted to investigate phylogenetic aspects in four genera of Penaeidae from the Arabian Sea with the aim to elucidate the evolutionary history of the major genera of the family. Results of this study would be used to test whether current taxonomic groupings are natural.

MATERIALS AND METHODS

Sampling: Samples of *Penaeus canaliculatus*, *P. indicus*, *Metapenaeus dobsoni*, *M. monoceros*, *Parapeneaeopsis stylifera* and *Trachypenaeus curvirotris* were collected from the coast of Mangalore in Arabian Sea. Samples transported in dry ice to laboratory, where they were identified morphologically as described by Dall *et al*,[17]. Pleopod muscles were
taken from shrimps and stored at -80 °C until further use.

2.2. Extraction of Enzymes: Adequate portions (250 mg) of tissue were first minced and homogenized using a glass homogenizer under cold condition. Using buffer containing sucrose (50%), 0.2 M Tris HCl (pH 7.2), EDTA (64 mg/100 mL). The homogenates were then centrifuged at 10,000 rpm for 20 min at 4°C. After the centrifugation, the supernatant was collected and used for further analyses.

Electrophoresis and Staining: Vertical polyacrylamide gel electrophoresis was used for the separation of allozymes at different enzyme loci. Gels consisted of 5% acrylamide and bis-acrylamide, and electrophoresis was run at 80 V at 4°C. The two buffers systems and three enzymes systems investigated are summarized in Table 1.

The bands of each enzyme were revealed by incubating the gels in the dark at 37°C in the presence of specific histochemical staining solution until sharp bands were visualized. The locus and allele designations were followed according to the standardized genetic nomenclature for protein-coding loci.

Results: Based on zymogram analysis as shown in Figures (3.1, 3.2 and 3.3), Nei's genetic identity (I) and genetic distance (D) were estimated for all species studied, and a matrix was produced as shown in Table 2. Based on genetic distance matrix (Table 2), a dendrogram was generated using UPGMA clustering method (Fig. 3.4). In addition, a neighbor-joining tree was also obtained using the same data set (Fig. 3.5). In both trees, the affinity of species, except Trachypeneaus curvirotris and Parapeneaus stylifera were supported by clustering as internal nodes. Penaeus spp. consistently formed the deepest branch of neighbor-joining tree with Parapeneaus, Trachypeneaus and Metapeneaus in the other branch. Although T. curvirotris is grouped with Metapeneaus clade in UPGMA tree, this species is grouped with Penaeus clade in neighbor-joining tree.

The topology of NJ tree differs from that of UPGMA only with phylogenetic positions of Trachypeneaus curvirotris and Parapeneaus stylifera. In UPGMA tree, T. curvirotris was clustered with Metapeneaus spp., while it was grouped with Penaeus in NJ tree. P. stylifera was grouped with Penaeus spp. in the UPGMA tree and does not cluster as internal node in the NJ tree. This clustering pattern suggests that Trachypeneaus is most likely a polyphyletic.

Discussion: As for the divergences of the various taxa in Penaeidae, there are two different opinions. Based on morphological features, Burkenroad divided the Penaeidae into three tribes: the Peneini (Penaeus, Heteropenaers, Funchalia, Pelagopenaeus), the Parapeneini (Parapeneaus, Artemesia, Penaeopsis, Metapeneaus), and the Trachypeneini (Metapeneaus, Macropetasma, Trachypeneaus, Atypopenaeus, Protrachypene, Xiphopenaeus, Parapeneaus, Trachypeneaus). While using similarity matrices computed on the basis of a complex classification of morphological features, Kubo distinguished Penaeidae as five groups: group 1 (Penaeus), group 2 (Penaeopsis), group 3 (Atypopenaeus, Trachypeneaus, Metapeneaus), group 4 (Parapeneaus, Parapeneaus, Trachypeneaus), and group 5 (Metapeneaus). Group 4 diverged a little earlier than the other four groups that diverged together.

The genetic identity, I, ranged in the two genera Penaeus and Metapeneaus, similar to the range of reported in the previous study of the two genera. A comparison of the I values between species included in both studies reveals that the corresponding values for M. dobsoni – M. monoceros pair differed by 0.02, yet for P. indicus-P. canaliculatus differs by 0.105. The differences in genetic similarity may be due to different enzymes used in the two studies. The differences may also partially be attributed to the genetic differentiation among populations of the same species which has been documented in some penaeids.

I values among species of 4 genera in the present study are within the range of 0.36-0.92 reported for decapod crustaceans. The highest I value of 0.59 between Parapeneaus stylifera and Trachypeneaus curvirotris, although not as high as the upper bound of the congeneric range as summarized by Hedgecock and Thorpe and Sole-Cava, suggest their affinity. The difference in external features of these two congeneric species such as length of rostrum is, however, quite large. In Trachypeneaus, the revision by Perez Farfante and Kensley is supported by the present data. In addition, present data show that Penaeus indicus and P. canaliculatus were grouped together. They are genetically more related than they are to the other genera studied. Nevertheless, these two species, formerly belonged to subgenera Marsupenaeus and Penaeus, respectively, were also placed into different genera in the scheme of Perez Farfante and Kensley. The recent revision for the Penaeus remains to be confirmed by other data. In Burkenroad's 3-tribe
Table 1: Allozymes screened and buffers systems

<table>
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<tr>
<th>Enzyme</th>
<th>Abbreviation and enzyme code</th>
<th>Buffer</th>
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<tr>
<td>Glucose-6-phosphate isomerase</td>
<td>PGI 5.1.3.9</td>
<td>Boric acid, NaOH buffer</td>
</tr>
<tr>
<td>Glutamat oxaloacetic transminase</td>
<td>GOT 2.6.1</td>
<td>Boric acid, NaOH buffer</td>
</tr>
<tr>
<td>Arginine phosphokinase</td>
<td>APK 2.7.3.3</td>
<td>Tris EDTA borate buffer</td>
</tr>
</tbody>
</table>

Table 2: Nei’s (1978) genetic distance (D, below diagonal) and genetic identity (I, above diagonal) among penaeid species. Penaeus canaliculatus (P. cana), P. indicus (P. indi), Metapenaeus dobsoni (M. dobs), M. monoceros (M. mono), Parapenaeopsis stylifera (Pa. styl) and Trachypeneaus curvirotris (T. curv)

<table>
<thead>
<tr>
<th></th>
<th>M. dobs</th>
<th>M. mono</th>
<th>Pa. styl</th>
<th>P. cana</th>
<th>P. indi</th>
<th>T. curv</th>
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</thead>
<tbody>
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<td>0.479</td>
<td>0.431</td>
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<tr>
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<td>0.922</td>
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</table>

Fig. 1: Zymogam of Glucose-6-phosphate isomerase (PGI), in Penaeidae. Lane 1: P. indicus, Lane 2: Penaeus canaliculatus Lane 3: Metapenaeus dobsoni Lane 4: M. monoceros Lane 5: Parapenaeopsis stylifera Lane 6: Trachypeneaus curvirotris

Fig. 2: Zymogam of Glutamat oxaloacetic transminase (GOT), in Penaeidae. Lane 1: P. indicus, Lane 2: Penaeus canaliculatus Lane 3: Metapenaeus dobsoni Lane 4: M. monoceros Lane 5: Parapenaeopsis stylifera Lane 6: Trachypeneaus curvirotris
Fig. 3: Zymogram of Arginine phosphokinase (APK), in Penaeidae. Lane 1: *P. indicus*, Lane 2: *Penaeus canaliculatus* Lane 3: *Metapenaeus dobsoni* Lane 4: *M. monoceros* Lane 5: *Parapeneaopsis stylifera* Lane 6: *Trachypeneaus curvirostris*

Fig. 4: Phylogenetic relationships among species of four penaeid genera based on UPGMA analysis of genetic distance given in Table 3.2.

Fig. 5: Phylogenetic relationships among species of four penaeid genera based on neighbor-joining analysis of genetic distance given in Table 3.2.

grouping scheme, tribe Peneini diverged earlier than other two tribes, while tribes Parapeneini and Trachypeneini shared a common ancestor. In Kubo’s\(^{[27]}\) 5-group scheme, *Parapeneaopsis* was grouped with *Trachypeneaus* and *Parapeneaopsis* in the group Parapeneaus which was believed to diverge earlier than other two groups, while *Metapenaeus* was included in the group Atypopenaeus (for comparison of the two phylogenetic schemes,). In the present study, the phylogenetic relationships among 4 penaeid genera were clearly demonstrated in UPGMA and neighbor-joining trees (Figs. 3.4 and 3.5). *Metapenaeus* and *Trachypeneaus* were grouped together in both trees.

The topology of NJ tree differs from that of UPGMA only with phylogenetic position of *Trachypeneaus curvirostris*. This discrepancy deserves some consideration. The UPGMA method holds an assumption of constant rate of evolution\(^{[28]}\) and finds the pair-species with the least distance as the starting node, followed by a sequential clustering procedure for the other species by taking arithmetic mean as the branch length\(^{[45]}\). Neighbor-joining employs a similar procedure but use a different algorithm and produces a rate-corrected pairwise distance matrix before an additive tree is produced\(^{[42]}\). The neighbor-joining method may be more robust against the effects of unequal evolutionary rates in different lineages and give better estimates of individual branch length\(^{[28]}\). In the present study the difference in the tree topology in the two tree-building methods may be due to the limitation of allozyme data in phylogenetic construction of highly divergent groups\(^{[1,24]}\). On the other hand, it may also be an indication of the heterogeneity of the presumptive phylogenetic grouping. Tribe Trachypeneini of Burkenroad\(^{[4]}\) which is composed of up to 8 genera, is thought to be less compact. Burkenroad\(^{[6]}\) stated that compared with other compact groupings; there were only one or two morphological characters that distinguished the members of tribe Trachypeneini from other tribes in
Penaeidae. Thus the unresolved phylogenetic position for *Trachypenaeus* may have revealed the real situation within this largest tribe. It seems that the phylogenetic relationship of this highly heterogeneous group deserves further examination using other data set, such as DNA sequence. Despite this uncertainty, present allozyme data resolves major branchings in Penaeidae, and supports the morphological phylogeny proposed by Burkenroad[6].

ACKNOWLEDGMENTS

We thank Dr. Dinesh Babu Director of CMFRI in Mangalore for helping in the collection and identifying of samples.

REFERENCES


