The molecular identification and phylogenetic reconstruction of Palaemonid and Penaeid shrimp from the southern part of Bangladesh

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Abstract. The study aimed to reveal the molecular identification and relationships between Palaemonid prawns and Penaeid shrimp in Bangladesh. Combined, they form an important economic part of tropical and subtropical fisheries. These species have a wide distribution range around the mangrove ecosystems, rivers, estuaries, flood plains, inundated natural depression water bodies, rice fields and other inland water bodies. Some of these species have a lack of information available about their biological and molecular characteristics. This study revealed the identification of prawn and shrimp species based on the molecular approach employed using mitochondrial COI gene markers, PCR, sequencing and matching the sequence to that stored in the NCBI database; four Palaemonid species (Macrobrachium rosenbergii, M. lamarrei, M. kistnense, Palaemonstiliferus) and one Penaeid shrimp (Penaeus monodon) were confirmed with 99-100\% confirmation of identity with multiple sequencing alignments. The inter-specific genetic divergence (K2P distance) among the family Palaemonid (Macrobrachium and Palaemon) is 0.171-0.257 \%, and intraspecific genetic distance was revealed to be 0.243-0.302\%. This result will be useful for obtaining the basic genetic information of these species and it will be helpful for future studies looking into the genetic biodiversity of the population structure, and finally, for the conservation and management of these resources in the southern part of Bangladesh.

1. Introduction
Bangladesh is a riverine country blessed with many rivers, canals, depressions, oxbow lakes, ponds, and floodplains, covering a huge area of water resources totaling about 4.70 million hectares [1]. In 2016, Bangladesh produced 2.20 million tons of fish from aquaculture and 1.05 million tons from inland freshwater [2, 3]. Shrimp aquaculture is one of the fastest growing financial activities in the coastal areas of Bangladesh and is the 5\textsuperscript{th} largest shrimp producer country in the world [3]. In Bangladesh, \textit{P. monodon} comprises 60\% of farmed shrimp production, followed by the giant freshwater prawn, \textit{Macrobrachium rosenbergii}, which accounts for 25\% of production. The remaining portion comes from other shrimp species including \textit{Metapenaeus monoceros}, \textit{Fenneropenaeus indicus}, \textit{Penaeus semisulcatus}, and \textit{F. merguiensis} [4, 5].
The giant freshwater prawn *Macrobrachium rosenbergii* is a commercially cultured important species of Palaemonid freshwater prawn in Bangladesh [6]. It is found throughout the tropical and subtropical areas of the Indo-Pacific region, from India to Southeast Asia and Northern Australia. This species has also been introduced to Asian countries (Thailand, China, Japan), Africa, New Zealand, the Americas and the Caribbean. It is one of the biggest freshwater prawns in the world and is widely cultivated in several countries for food [7]. A sub-tropical climate and a vast area of water bodies provide a unique opportunity for the production of *Macrobrachium* spp. Twenty-four species of freshwater prawns, including 10 species of *Macrobrachium*, are found in Bangladesh [8].

We examined the phylogenetic relationship of four Palaemonid (*Macrobrachium rosenbergii, M. lamarrei, M. kistnense*, and *Palaemonstyliferus*) and one Penaeid shrimp (*Penaeus monodon*) species collected from the southern part of Bangladesh in March 2017. These species have a wide range of distribution around the mangrove ecosystem, including in rivers, estuaries and other inland water bodies, but there is a lack of information about its biological and molecular characteristics. Therefore, molecular identification and the phylogenetic reconstruction of the prawns and shrimps of Bangladesh will be helpful to improving the genetic information available for the future conservation management of this fishery resource.

2. Materials and methods

2.1. Sample collection and preparation

Ten (10) samples were collected from the local fish market, Khulna (22°50′44.3″N 89°32′27.7″E), in the southern part of Bangladesh. After the collection of all species was undertaken, morphological identification was done according to [9, 10]. Digital photographs of all of the samples were taken, and then the specimens were directly preserved in 96% ethanol and deposited at the Department of Fisheries headquarter in Dhaka, Bangladesh.

2.2. Samples collection

Tissue samples were taken from the abdominal region and genomic DNA was extracted using the DNeasy® Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer’s instructions. In brief, 200 mg of tail muscle was dissected and mixed with 1x lysis buffer, which was further homogenized by the Tissue Lyser II motorized homogenizer (QIAGEN, Hilden, Germany). The quantification of the purified genomic DNA was performed using the NanoDrop spectrophotometer, ND-1000 (Thermo Scientific, Waltham, MA, USA). The extracted genomic DNA was kept at -20°C for further analysis.

2.3. PCR amplification

One pair of universal primer sets, LCO1490 and HCO2198 [11], were used to obtain the mitochondrial cytochrome c oxidase I (COI) partial gene sequences. The PCR mixture (20µL) contained 11.2 µL ultrapure water, 1 µL of each of the primers (0.5 µM, forward and reverse), 0.2 µL Ex Taq DNA polymerase (TaKaRa Bio Inc. Japan), 2.0 µL 10X Ex Taq buffer, a 2.0 µL deoxynucleotide triphosphate (dNTPs) mixture (2.5 mM, TaKaRa, Japan), and 2.0 µL genomic DNA as the template. PCR was performed under the following settings: the initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec and with an extension at 72°C for 45 sec. The PCR products were separated by 1.5 % of agarose gel and electrophoresis after staining with the loading star (Dynebio, Sungnam, Republic of Korea). The PCR products of the COI were purified using the AccuPrep®Gel purification kit according to the manufacturer’s protocol (Bioneer, Korea).
Table 1. Species list with the GenBank accession number for the Palaemonid prawn and Penaeid shrimps and their cytochrome c oxidase subunit I (COI) genes

| ID No. | Species name            | Locality   | GenBank no. | References                      |
|--------|-------------------------|------------|-------------|---------------------------------|
| SH 01  | *Penaeus monodon*       | Bangladesh | MH884751    | In this study                   |
| SH 02  | *Macrobrachium rosenbergii* | Bangladesh | MH884752    | In this study                   |
| SH 05  | *Macrobrachium lamarrei* | Bangladesh | MH884753    | In this study                   |
| SH 07  | *Palaemonstyliferus*    | Bangladesh | MH884754    | In this study                   |
| SH 10  | *Macrobrachium kistnense* | Bangladesh | MH884755    | In this study                   |
|        | *Penaeus monodon*       | India      | KX399427    | Ram et al. (20016)             |
|        | *Macrobrachium rosenbergii* | India      | MF563570    | Mandal et al. (2017)           |
|        | *Macrobrachium lamarrei* | Bangladesh | MF621334    | Habib et al. (2017)            |
|        | *Palaemonstyliferus*    | Bangladesh | MF621340    | Habib et al. (2017)            |
|        | *Macrobrachium kistnense* | India      | KY451617    | Deepa and Karuthapandi (2017)  |
|        | *Scylla paramamosain*   | China      | AY750937    | Ma et al. (2006)               |

3. Result and discussion

The LCO and HCO primer set was amplified for five samples and the total length of the aligned mitochondrial COI gene sequence was from 508-634 base-pairs (bp). The average nucleotide compositions were; *Macrobrachium rosenbergii* with T 26.8%, C 25.5%, A 28.5%, and G 19.3%; *M. lamarrei* with T 28.3%, C 25.9%, A 26.1%, and G 19.7%; *M. kistnense* with T 29.8%, C 21.8%, A 30.4% and G 18.0%; *Palaemonstyliferus* with T 31.3%, C 23.8%, A 26.1%, and G 18.8% and *Penaeus monodon* with T 36.0%, C 17.8%, A 27.4%, and G 18.8% respectively. The interspecific genetic divergence (K2P distance) in the family Palaemonid (*Macrobrachium* and *Palaemon*) was 0.171 - 0.257% and the intraspecific genetic distance revealed was 0.243 - 0.302% (Table 2). The COI region sequences were compared to the references from the NCBI database and 99 to 100% of the identity queries were covered. All of the sequences were submitted to the GenBank database to improve the COI region information of the shrimp species collected from Bangladesh waters (Table 1).

The base composition of the COI gene region varied among the species. The giant tiger prawn (Sh01), *Penaeus monodon* had a 36.46% GC content similar to the *P. Monodon* species from India, with 36.46% (628bp). *M. rosenbergii* and *M. lamarrei* had a similar GC% of 45.55% (630bp) and 46.06% (508bp) respectively. *M. lamarrei*’s AT% and GC% showed different results compared to the previous research in India, which had a lower GC% from 40.0 - 40.7% [13]. However, two species, *M. kistnense* (40.95%; 608bp) and *Palaemonstyliferus* (42.355; 543bp) were lower than the references of 41.44 (608bp) and 43.27 (543 bp) respectively. This condition shows there to be a positive correlation in the genetic distance of both of species (Table 2)
Table 2. Genetic distance for the pair-wise nucleotide K2P divergence of the mitochondrial COI sequences among the samples with references.

| Species Name | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sh01 Penaeus monodon | 0.000 |       |       |       |       |       |       |       |       |       |
| KX399427 Penaeus monodon |       | 0.284 | 0.284 |       |       |       |       |       |       |       |
| Sh02 Macrobrachiumrosenbergii |       |       | 0.284 | 0.284 | 0.000 |       |       |       |       |       |
| MF653570 Macrobrachiumrosenbergii |       | 0.284 | 0.284 | 0.000 |       |       |       |       |       |       |
| Sh05 Macrobrachiumlamarrei |       |       | 0.302 | 0.302 | 0.211 | 0.211 |       |       |       |       |
| MF621334 Macrobrachiumlamarrei |       | 0.302 | 0.302 | 0.211 | 0.211 | 0.000 |       |       |       |       |
| Sh07 Palaemonstyliferus |       |       | 0.276 | 0.276 | 0.257 | 0.257 | 0.294 | 0.294 |       |       |
| MF621340 Palaemonstyliferus |       | 0.290 | 0.290 | 0.267 | 0.267 | 0.291 | 0.291 | 0.291 | 0.011 |       |
| Sh10 Macrobrachiumkistnense |       | 0.243 | 0.243 | 0.171 | 0.171 | 0.215 | 0.215 | 0.274 | 0.287 |       |
| KY451617 Macrobrachiumkistnense |       | 0.252 | 0.252 | 0.174 | 0.174 | 0.234 | 0.234 | 0.271 | 0.284 | 0.018 |
| AY750937 Scylla paramamosain |       |       |       |       | 1.081 | 1.081 | 1.242 | 1.242 | 1.223 | 1.223 |
| Penaeus monodon | 1.262 | 1.293 | 1.223 | 1.223 | 1.262 | 1.293 |       |       |       |       |

The predicted phylogenetic information has been presented in Figure 1 in a phylogenetic tree (ML and NJ). It shows that all Macrobrachium and Palaemon styliferus were clustered in the same family [14] and that there was a close relationship to the Penaeid shrimp. The phylogenetic trees (NJ and ML) helped the researcher to figure out that the shrimp and prawns had a similar ancestry, but that they were clustered in different family groups (Penaeid and Palaemoid). Even though the BLASTN system confirmed SH10 as Macrobrachium kistnense, this sequence is not completely similar to the similar species from India. The interspecific distance between both species is 0.018, so SH10 is Macrobrachium kistnense, possibly as a Bangladeshi haplotype. Palaemon styliferus, from the GenBank database, also has an interspecific genetic distance of 0.011 to a similar species, P. styliferus. This species (MF621340) was collected from the Sundarbans area in Bangladesh. Beside P. styliferus, Penaeus monodon is a well-known species and found in the Sundarbans area, which is a world heritage site [15].

Figure 1. (a) Phylogenetic analysis based on the Maximum Likelihood (ML) shows that all species shared their habitat in both Bangladesh and Indian waters. (b) Phylogenetic analysis based on Neighbor- Joining (NJ) shows that all species shared their habitat in Bangladesh and Indian waters.

Molecular identification of the Palaemonid and Penaeid shrimp samples was successfully performed on five samples and the species were known to be Macrobrachiumrosenbergii, M. lamarrei, M. kistnense, Palaemonstyliferus; and Penaeus monodon. The phylogenetic analysis (ML and NJ) showed that all species share a habitat with P. monodon from India (Figure 1a, 1b). The natural range of this particular Palaemoid species is eastward from eastern Pakistan up to Borneo and Java (Wow or and Ng 2007, as M.dacqueti). The species is widely cultured both within its natural range and far
by beyond (Africa, South America). Further specimens have been found in Sao Paulo state, but these may represent a non-breeding population.

The current fishery product intensification program continues to be developed, which has made the Macrobrachium rosenbergii species the main export product of Bangladesh to the United States, Japan, the EU, the United Kingdom, Italy, Belgium and Germany. The giant prawn shrimp has developed a monoculture [16] or polyculture system[17]. From the results of the molecular identification, it can be determined that Macrobrachium rosenbergii is scattered in the southern region of Bangladesh. It was found that this type of giant freshwater prawn has a kinship with the same species in India which belongs to the M. rosenbergii region in the West. It is now known M. rosenbergii has three different distributions, namely Eastern, Western and Australian 'Race', separated by Huxley's and Wallace's lines [18].

Beside M. rosenbergii, another freshwater prawn, M. lamarrei also, is found in India [13] and distributed in the Indo West-Pacific region. It can also be found in Nepal [19], which have a part of their life cycle only in freshwater [20]. The previous study found M. lamarrei in the northeast and southwest part of Bangladesh [21]. Even though M. rosenbergii was a common species for prawn culturing in Bangladesh, another species is the wild freshwater prawn. Currently, M. lamarrei and M. kistnense are wild freshwater prawns and are not yet cultured for commercial purposes. They are very famous in artisanal fisheries in Bangladesh, reaching 93%, and only 7% have been found in industrial fisheries [22]. Palaemon styliferus is an estuarine prawn species commonly found as an artisanal fishery product in Bangladesh. Currently, there have been a very limited number of publications regarding the population and genetic study of this species. This species can be found in Sangu River, including 127 Ichthyofauna [23].

4. Conclusion

Molecular identification has successfully identified four species from the Palaemonid family and one Penaeid shrimp, collected from the southern part of Bangladesh. This identification clarified their phylogeographic status, and also improved the molecular information available in the GenBank database, which is very useful for genetic population studies and related topics. The phylogenetic analysis also showed the diversity and evolution of the five species of the Palaemonidae and Penaeidae family, distributed in Indo-Pacific waters (Bangladesh, India, Pakistan, Myanmar, Nepal etc).

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