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# DNA Barcoding of Freshwater Prawn Species of Two Genera *Macrobrachium* and *Caridina* Using mt-COI Gene

**Udayasuriyan R, Saravana Bhavan P* and Kalpana R**

## Abstract

**Objective:** The aim was to barcoding of *Macrobrachium* and *Caridina* species through mt-COI gene, and phylogenetic reconstruction based on the degree of genetic variability between species of these two genera.

**Methods:** Freshwater prawns were collected from few locations in the Cauvery River, Tamil Nadu, India. They were morphologically identified as *Macrobrachium lamarrei, Macrobrachium lamarrei lamarroids, Macrobrachium malcolmsonii* and *Caridina gracilipes*. Genomic DNA was isolated and amplification of mt-COI gene was done. The nucleotide divergence and some phylogenetic information were calculated by using MEGA v.6.01 and DAMBE. The phylognetic tree topology were reconstructed by Maximum likelihood model.

**Results:** The mt-COI gene sequences of these species showed 99-100% similarity. When *Macrobrachium* species were compared within themselves, they showed higher number of variable amino acid sites (539), which revealed some distance. When *Macrobrachium* species were compared with *Caridina*, they showed still higher number of variable amino acid sites (584), which revealed clear discrimination. In the subjected category, the mean value of inter-general divergence was more (6.934%) than that of the intra-general divergence (within *Macrobrachium* species, 3.628%; within *M. lamarrei*, 3.132%; within *Caridina*, 2.697%). When both subjected and retrieved species of these two genera were pooled together, the mean inter-general divergence value was also more (3.260%) than that of the intra-general divergence (*Macrobrachium*, 2.080%; within *M. lamarrei*, 1.222%; within *Caridina*, 2.200%). Therefore, the sequences of these two genera are more conserved as they showed 3.260-6.934% mean divergence or less subjected to evolutionary forces.

**Conclusion:** The species of *Macrobrachium*, and *Caridina* are clearly delineated from each other as the phylogenetic information obtained through mt-COI partial gene sequences are more conserved and less subjected to evolutionary forces, and thus, their species are genetically distinct.

**Keywords**

Mitochondrial COI Gene; *Macrobrachium*; *Caridina*; Divergence; Phylogenetic information; Tree topology

## Introduction

The Cauvery River in India, contributes a considerable amount of both fin and shellfish in states of Tamil Nadu and Karnataka. Regarding diversity of *Macrobrachium*, seven species, such as *Macrobrachium malcolmsonii*, *Macrobrachium nobilii*, *Macrobrachium scabriculum*, *Macrobrachium lamarrei*, *Macrobrachium rude*, *Macrobrachium australe* and *Macrobrachium aemulum* have been reported in this river [1]. Among them *M. malcolmsonii* is the most widely distributed and holds first place in capture and culture fisheries. The presence of *Caridina gracilipes*, *Macrobrachium malcolmsonii* and *Macrobrachium lamarrei* in this river have been reported recently [2].

The phylogenetic affinities among *Macrobrachium* species are poorly understood. Pereira [3] carried out the first phylogenetic study based on morphological characters of the family Palaemonidae. Liu et al. [4] carried out molecular taxonomy of *Macrobrachium*. Baker et al. [5] highlighted the presence of several cryptic lineages in Australian Paratya (Atyidae), some of which represented as true species through DNA barcoding. Species discrimination has also been studied genetically in *Caridina* and cingeners from potential source populations throughout the west Indo-West Pacific region [6]. The nucleotide substitution is the main driving force for the formation of new species. The literature depict that the DNA sequence has been used to investigate the phylogenetic and biogeographic relationship among *Macrobrachium canarae* and *Caridina gracilirostris* [7]; the morphometric and meristic characters along with DNA bar-coding of CytB gene sequence has been used to discriminate *Macrobrachium abrahami* sp. [8]; the morphological, and partial sequence of mitochondrial COI gene (mt-COI) has been used to resolve the taxonomic identity of *Caridina africana* in the Cape Floristic Region of South Africa [9]; the mt-COI gene has also been used for phylogeography and population genetic studies of many freshwater shrimp species, due to the existence of high levels of sequence variability [10-12]; species discrimination in Penaeid shrimps, *Macrobrachium*, crabs and planktons through mt-COI gene have also been reported by us recently [2,13-17].

The *time-series functions predict the prawn* divergence. This is prevailing in organisms which undergo ontogenetic habitat shifts, and where the potentially limiting resources are age-specific, that is different life stages limited by different types of resources [18]. In some species, density dependence can particularly be strong during the early juvenile stage [19-21], whereas in others, later stages are more heavily influenced by density [20,22-25]. Such divergent patterns in the ontogenetic timing of density dependence may also exist among different populations within the same species [26,27].

Generally, the presence of plastic characters in the genus *Macrobrachium* makes the accurate determination of species more difficult and problematic, and the phylogenetics studies were poorly understood. These were shorted-out in this study while sequencing using COI gene, and have predicted some phylogenetic information led to well resolved tree topology. Actually, the present study describes the degree of genetic variability between *Caridina* and *Macrobrachium* species through mt-COI gene. Furthermore, the sequence similarity, amino acid residues, sequence divergence and phylogenetic information, such as synonymous and non-synonymous

***Corresponding author:** Saravana Bhavan Periyakali, Department of Zoology, Bharathiar University, Coimbatore-641046, Tamil Nadu, India, Tel: +91-422-2428495; Fax: +91-422-2425706; E-mail: bhavan@buc.edu.in

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substitutions, transitional and transvertional substitutions, saturations and phylogenetic tree topology have also been studied.

## Materials and Methods

Freshwater prawn species were collected from Hogenakkal (12.11° N, 77.77° E), Kooduthurai (11.09° N, 78.05° E), Aliyar Dam (10.48 N, 76.96 E) and Karungulam (10.35° E, 79.40° N). They were identified based on morphological characters, such as overlapping of the second segment over first and third segments, rostral structure, rostral teeth, periopods and telson with the help of experts, Mr. M. Kathirvel, Former Principal Scientist, Central Institute of Brackish water Aquaculture, ICAR, Chennai, India, and Mrs. K. Valarmathi, Scientist C, ZSI, Chennai, India. The species collected from Hogenakkal were morphologically identified as *Macrobrachium lamarrei* and *Macrobrachium lamarrei lamarroids*. The species collected from Kooduthurai were morphologically discriminated as *Macrobrachium lamarrei* and *Caridina gracilipes*. The species collected from Aliyar Dam were also morphologically discriminated as *Macrobrachium lamarrei* and *Caridina gracilipes*. Whereas, the species collected from Karungulum was morphologically identified as *Macrobrachium malcolmsonii*.

Genomic DNA was isolated from the abdominal tissue by using Qiagen DNeasy Blood and Tissue Kit (Germany). 1% Agarose Gel Electrophoresis were performed and the genomic DNA was detected in a Gel documentation system. DNA amplification of mt-COI gene was carried out with universal primers of forward and reverse in natures, COIa and COIf respectively. These primer sets were worked well with freshwater prawns [2]. The amplified product was resolved with 2% AGE, and they were sequenced. The forward and reverse sequences were aligned pairwise by using CAP3. The sequence similarity available with NCBI database was identified by BLAST. The internal stop codon was removed by using BLAST. The reading frame shift was detected by ORF finder. The trimmed sequence was authenticated with GenBank.

The multiple sequence alignment was done by using T-Coffee and the aligned sequence were highlighted with multiple align show (MAS) as identical, similar and variable sites of amino acids. The nucleotide composition (AT and GC bias), nucleotide divergence (K2P model) [28] and some phylogenetic information were calculated by using MEGA v.6.01. Assessment of synonymous (Ks) and non-synonymous (Ka) substitutions for 3rd codon positions was calculated by Li93 method of DAMBE [29]. Similarly, the synonymous (Ka) and non-synonymous (Ks) substitutions for 3rd codon positions was predicted by Muse-Gaut model of codon substitution [30]. The transitional (Ts) and transvertional (Tv) substitutions was determined by the Felsenstein model of nucleotide substitution [31]. Analysis of sequence saturation, index of substitutional saturation (Iss) and critical value of index of substitutional saturation (Iss.c) was done by Xia method using DAMBE [32,33]. Finally the phylogenetic tree was reconstructed by Maximum Likelihood model [34,35].

## Results and Discussion

The isolated genomic DNA was confirmed as >10 kb and its amplified products showed ~650 bp (Figures 1 and 2). The actual length of the trimmed sequences were 443, 587, 657, 616, 598, 654 and 657 bp, for *M. lamarrei* (Hogenakkal), *M. lamarrei* (Kooduthurai), *M. lamarrei* (Aliyar Dam), *M. lamarrei lamarroids* (Hogenakkal), *M. malcolmsonii* (Karungulam), *C. gracilipes* (Kooduthurai) and *C. gracilipes* (Aliyar Dam) respectively, which were authenticated with GenBank (KX214617, KX788818, KX214618, KX788820, MF838938, KX788819 and KX214619 respectively).

The BLAST results for these sequences showed 99-100% similarity with the retrieved sequences from the NCBI database. The similarity between the sequences usually depend entirely on the similarity in nucleotide frequencies, which is based on level of substitutional saturation, which in turn decreases phylogenetic information [32]. The results of multiple sequence alignment revealed the following, when *Macrobrachium* species were compared within themselves showed more number of variable amino acid sites (539). However, when *Macrobrachium* species were compared with *Caridina*, they showed still higher number of variable amino acid sites (584). Instead, *Caridina* species taken from two different locations showed more number of identical amino acid residues (654) and very less numbers of variable amino acid (4). Therefore, *Caridina* species showed a very close relationship within themselves, which indicates the fact that there was no sequential variations appeared even though they were taken from two different locations. Whereas, *Macrobrachium* species showed some distance within themselves because of more number of variable amino acids. Moreover, *M. lamarrei* taken from three locations also showed a higher number of variable amino acids (390)

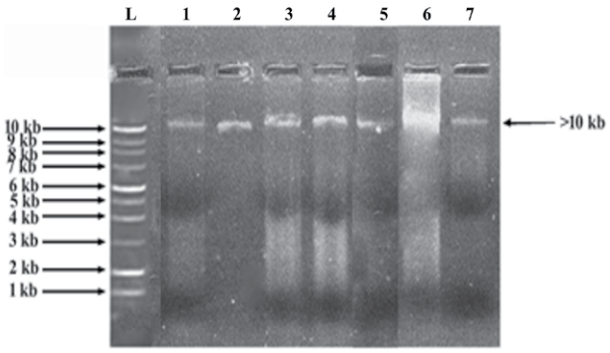


**Figure 1:** 1% AGE showed >10 kb of genomic DNA.

**L**-1 kb ladder; **1**-*Macrobrachium lamarrei* (Hogenakkal); **2**-*Macrobrachium lamarrei* (Kooduthurai); **3**-*Macrobrachium lamarrei* (Aliyar Dam); **4**-*Macrobrachium lamarrei lamarroids* (Hogenakkal); **5**-*Macrobrachium malcolmsonii* (Karungulam); **6**-*Caridina gracilipes* (Kooduthurai); **7**-*Caridina gracilipes* (Aliyar Dam).

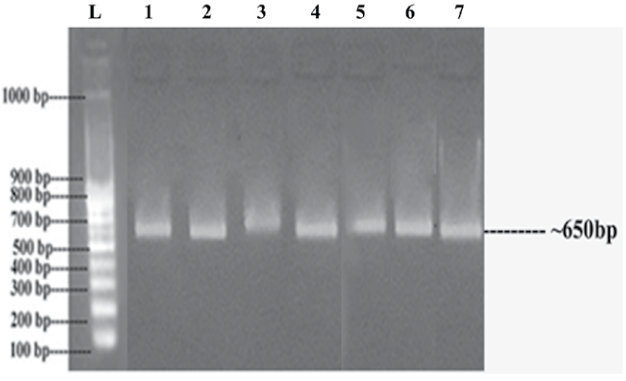


**Figure 2:** 2% AGE showed ~650 bp amplified product of mt-COI gene.

**L**-100 bp ladder; **1**-*Macrobrachium lamarrei* (Hogenakkal); **2**-*Macrobrachium lamarrei* (Kooduthurai); **3**-*Macrobrachium lamarrei* (Aliyar Dam); **4**-*Macrobrachium lamarrei lamarroids* (Hogenakkal); **5**-*Macrobrachium malcolmsonii* (Karungulam); **6**-*Caridina gracilipes* (Kooduthurai); **7**-*Caridina gracilipes* (Aliyar Dam).

**Table 1:** Nucleotide base composition percentage in COI partial gene sequences of subjected *Macrobrachium* and *Caridina* species.

| Species name | Nucleotide % | | | | | |
|---|---|---|---|---|---|---|
| | A | T | AT | G | C | GC |
| *Macrobrachium lamarrei* of Hogenakkal | 30.9 | 28.7 | 59.6 | 27.5 | 12.9 | 40.4 |
| *Macrobrachium lamarrei* of Kooduthurai | 29.3 | 30.0 | 59.3 | 17.2 | 23.5 | 40.7 |
| *Macrobrachium lamarrei* of Aliyar Dam | 30.0 | 30.0 | 60.0 | 17.0 | 23.0 | 40.0 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal | 33.8 | 28.9 | 62.7 | 21.4 | 15.9 | 37.3 |
| *Macrobrachium malcolmsonii* of Karungulam | 27.9 | 27.4 | 55.4 | 26.9 | 17.7 | 44.6 |
| *Caridina gracilipes* of Kooduthurai | 30.1 | 29.7 | 59.8 | 22.9 | 17.3 | 40.2 |
| *Caridina gracilipes* of Aliyar Dam | 30.0 | 29.8 | 59.8 | 23.0 | 17.2 | 40.2 |
| Within *Macrobrachium* species | 30.4 | 29.0 | 59.4 | 22.0 | 18.6 | 40.6 |
| Within *Caridina gracilipes* | 30.0 | 29.8 | 59.8 | 23.0 | 17.2 | 40.2 |
| Between *Macrobrachium* and *Caridina* species | 30.3 | 29.2 | 59.5 | 22.1 | 18.4 | 40.5 |
| Within *M. lamarrei* | 30.0 | 29.5 | 59.6 | 20.5 | 19.8 | 40.3 |

**COI**-Cytochrome C oxidase subunit I gene; **A**-Adenine; **T**-Thymine; **G**-Guanine; **C**-Cytosine

**Table 2:** Nucleotide divergence of *Macrobrachium* and *Caridina* species.

| Comparisons | Divergence (%) |
|---|---|
| **Within *Macrobrachium* species (subjected)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Kooduthurai | 2.321 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Aliyar Dam | 1.707 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei lamarroids* of Hogenakkal | 1.182 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* of Karungulam | 2.583 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei lamarroids* of Hogenakkal | 1.182 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* of Aliyar Dam | 5.370 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* of Karungulam | 2.583 |
| *Macrobrachium lamarrei lamarroids* of Hoganakkal vs. *Macrobrachium lamarrei* of Aliyar Dam | 0.701 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium lamarrei* of Aliyar Dam | 15.363 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* of Karungulam | 3.293 |
| **Mean Divergence** | 3.628 |
| **Within *Caridina* species (subjected)** | |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* of Aliyar Dam | 2.697 |
| **Between *Macrobrachium* and *Caridina* species (subjected)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* of Kooduthurai | 1.026 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Caridina gracilipes* of Kooduthurai | 5.990 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* of Kooduthurai | 23.268 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* of Kooduthurai | 3.074 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* of Kooduthurai | 1.068 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* of Aliyar Dam | 1.026 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Caridina gracilipes* of Aliyar Dam | 5.990 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* of Aliyar Dam | 23.268 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* of Aliyar Dam | 3.564 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* of Aliyar Dam | 1.068 |
| **Mean Divergence** | 6.934 |
| **Within *M. lamerrei* (subjected)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Kooduthurai | 2.321 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Aliyar Dam | 1.707 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* of Aliyar Dam | 5.370 |
| **Mean Divergence** | 3.132 |
| **Within *Macrobrachium* species (subjected and retrieved)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* (India) | 0.291 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* (India) | 0.678 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium lamarrei* (India) | 2.697 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium lamarrei* (India) | 2.813 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium lamarrei* (India) | 2.665 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 0.288 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* (Karala, India) | 2.813 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 2.665 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Karala, India) | 3.583 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium malcolmsonii* (India) | 2.697 |

| | |
|---|---|
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Karala, India) | 0.128 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 3.023 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium malcolmsonii* (Kerala, India) | 4.200 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* (India) | 0.620 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium malcolmsonii* (India) | 0.706 |
| *Macrobrachium malcolmsonii* (Tamil Nadu, India) vs. *Macrobrachium lamarrei* (India) | 2.665 |
| *Macrobrachium malcolmsonii* (Kerala, India) vs. *Macrobrachium lamarrei* (India) | 4.200 |
| *Macrobrachium malcolmsonii* (India) vs. *Macrobrachium malcolmsonii* (India) | 0.706 |
| **Mean Divergence** | 2.080 |
| **Within *Caridina* species (subjected and retrieved)** | |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* (India) | 2.260 |
| *Caridina gracilipes* of Aliyar Dam vs. *Caridina gracilipes* (China) | 2.260 |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* (India) | 2.126 |
| *Caridina gracilipes* of Aliyar Dam vs. *Caridina gracilipes* (China) | 2.126 |
| *Caridina gracilipes* (India) vs. *Caridina gracilipes* (China) | 1.733 |
| **Mean Divergence** | 2.200 |
| ***Macrobrachium* and *Caridina* species (subjected and retrieved)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Kooduthurai | 2.321 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* of Aliyar Dam | 1.707 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei lamarroids* of Hogenakkal | 1.182 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* of Karungulam | 2.583 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei lamarroids* of Hogenakkal | 1.182 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* of Aliyar Dam | 5.37 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* of Karungulam | 2.583 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium lamarrei lamarroids* of Hogenakkal | 0.701 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium malcolmsonii* of Karungulam | 15.363 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* of Karungulam | 3.293 |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* of Aliyar Dam | 2.697 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* of Kooduthurai | 1.026 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Caridina gracilipes* of Kooduthurai | 5.99 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* of Kooduthurai | 23.268 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* of Kooduthurai | 3.074 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* of Kooduthurai | 1.068 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* of Aliyar Dam | 1.026 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Caridina gracilipes* of Aliyar Dam | 5.99 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* of Aliyar Dam | 23.268 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* of Aliyar Dam | 3.564 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* of Aliyar Dam | 1.068 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* (India) | 0.291 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* (India) | 0.678 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium lamarrei* (India) | 2.697 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium lamarrei* (India) | 2.813 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium lamarrei* (India) | 2.665 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 0.288 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* (Kerala, India) | 2.813 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 2.665 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Kerala, India) | 3.583 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 2.697 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Kerala, India) | 0.128 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 3.023 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium malcolmsonii* (Kerala, India) | 4.200 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Macrobrachium malcolmsonii* (Tamil Nadu, India) | 0.620 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Macrobrachium malcolmsonii* (Kerala, India) | 0.706 |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* (India) | 2.26 |
| *Caridina gracilipes* of Aliyar Dam vs. *Caridina gracilipes* (China) | 2.26 |
| *Caridina gracilipes* of Kooduthurai vs. *Caridina gracilipes* (India) | 2.126 |
| *Caridina gracilipes* of Aliyar Dam vs. *Caridina gracilipes* (China) | 2.126 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* (India) | 0.024 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Caridina gracilipes* (China) | 3.561 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* (India) | 4.926 |

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|---|---|
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* (India) | 0.823 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* (India) | 0.945 |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Caridina gracilipes* (China) | 0.500 |
| *Macrobrachium lamarrei* of Kooduthurai vs *Caridina gracilipes* (China) | 4.309 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Caridina gracilipes* (China) | 3.460 |
| *Macrobrachium lamarrei lamarroids* of Hogenakkal vs. *Caridina gracilipes* (China) | 1.313 |
| *Macrobrachium malcolmsonii* of Karungulam vs. *Caridina gracilipes* (China) | 4.587 |
| *Macrobrachium lamarrei* (India) vs *Caridina gracilipes* of Kooduthurai | 1.987 |
| *Macrobrachium lamarrei* (India) vs. *Caridina gracilipes* of Aliyar Dam | 1.063 |
| *Macrobrachium malcolmsonii* ( India) vs. *Caridina gracilipes* of Kooduthurai | 1.572 |
| *Macrobrachium malcolmsonii* (India) vs. *Caridina gracilipes* of Aliyar Dam | 1.572 |
| *Macrobrachium malcolmsonii* (India) vs. *Caridina gracilipes* of Kooduthurai | 2.504 |
| *Macrobrachium malcolmsonii* (India) vs. *Caridina gracilipes* of Aliyar Dam | 2.504 |
| **Mean Divergence** | 3.260 |
| **Within *M. lamerrei* (subjected and retrieved)** | |
| *Macrobrachium lamarrei* of Hogenakkal vs. *Macrobrachium lamarrei* (India) | 0.291 |
| *Macrobrachium lamarrei* of Kooduthurai vs. *Macrobrachium lamarrei* (India) | 0.678 |
| *Macrobrachium lamarrei* of Aliyar Dam vs. *Macrobrachium lamarrei* (India) | 2.697 |
| **Mean Divergence** | 1.222 |

**Table 3:** Overall average phylogenetic information of subjected and retrieved *Macrobrachium* and *Caridina* species.

| **Phylogenetic information** | | **Ks** | **Ka** | **Ks-Ka** | **Ts** | **Tv** | **Tv-Ts** | **Iss** | **Iss.c** | **Iss.c-Iss** |
|---|---|---|---|---|---|---|---|---|---|---|
| Subjected species alone | *Macrobrachium* | 0.739 | 2.181 | 1.442 | 0.26 | 0.37 | 0.11 | 0.916 | 0.79 | 0.126 |
| | *Macrobrachium* and *Caridina* | 0.719 | 2.178 | 1.459 | 0.27 | 0.40 | 0.13 | 0.768 | 0.886 | 0.118 |
| | *M. lamarrei* | 0.761 | 2.165 | 1.404 | 0.23 | 0.33 | 0.10 | 0.760 | 0.812 | 0.052 |
| Subjected and retrieved species | *Macrobrachium* | 0.661 | 2.235 | 1.574 | 0.28 | 0.41 | 0.13 | 0.777 | 0.935 | 0.158 |
| | *Caridina* | 0.665 | 2.238 | 1.573 | 0.24 | 0.36 | 0.12 | 0.702 | 0.806 | 0.104 |
| | *Macrobrachium* and *Caridina* | 0.693 | 2.210 | 1.517 | 0.29 | 0.43 | 0.14 | 0.756 | 1.062 | 0.306 |
| | *M. lamarrei* | 0.704 | 2.201 | 1.497 | 0.29 | 0.39 | 0.10 | 0.615 | 0.805 | 0.190 |

**Ks**-Synonymous substitution; **Ka**-Non-synonymous substitution; **Ts**-Transitional substitution; **Tv**-Transversional substitution; **Iss**-Index of substitution saturation; **Iss.c**-Critical value of index of substitution saturation

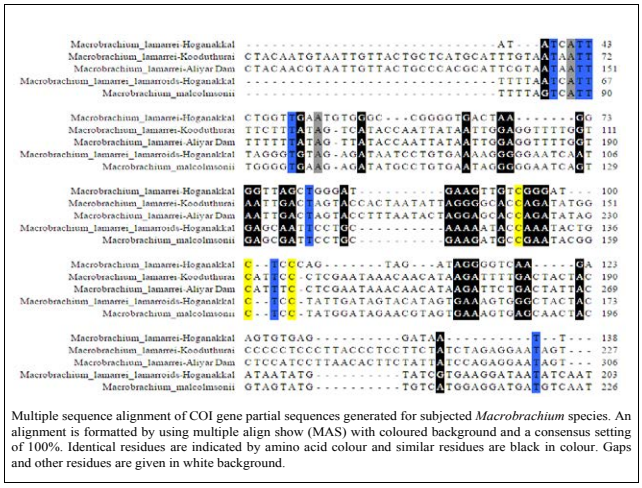
than that of identical amino acid (202), which indicates the fact that sequential variations occurred in the same species due to locality variation (Plate 1). At the outset, when the species of two genera, *Macrobrachium* and *Caridina* were compared, they showed much more variation in the amino acid sites, which indicate the fact that they are very well discriminated.

## Nucleotide base composition

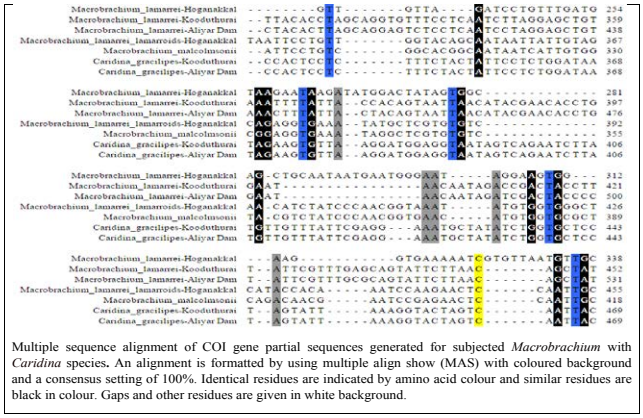
The base composition of the COI gene fragment varied among the species, AT biases was ranged from 55.4% to 62.7% (*M. malcolmsonii* and *M. lamarrei lamarroids* respectively) and the GC bias ranged between 37.3-44.6% (*M. lamarrei lamarroids* and *M. malcolmsonii*). The *Macrobrachium* species were compared within themselves showed 59.4% (A=30.4; T=29.0) and 40.6% of GC (G=22.0; C=18.6). The *Caridina* species were compared within themselves showed 59.8% AT bias (A=30; T=29.8) and 40.2% of GC (G=23; C=17.2). When *Macrobrachium* and *Caridina* were compared, they showed 59.5% AT bias (A=30.3; T=29.2) and 40.5% of GC (G=22.1; C=18.4). *M. lamarrei* within themselves showed 59.6% AT bias (A=30; T=29.5) and 40.3% of GC (G=20.5; C=19.8). In these four categories, collectively, the AT biases (60%) were more than that of the GC biases (40%). Therefore, no differences were seen in AT and GC biases between both genera. The higher AT bias recorded indicates the lower abundance of nuclear copies of mt-DNA (NUMTs) genes known as pseudogenes, homologs or paralogs in both genera (Table 1). The higher AT biases have also been reported by us in marine crabs [14], freshwater crabs and prawns [2,15] and freshwater plankton [16,17].

## Nucleotide divergence

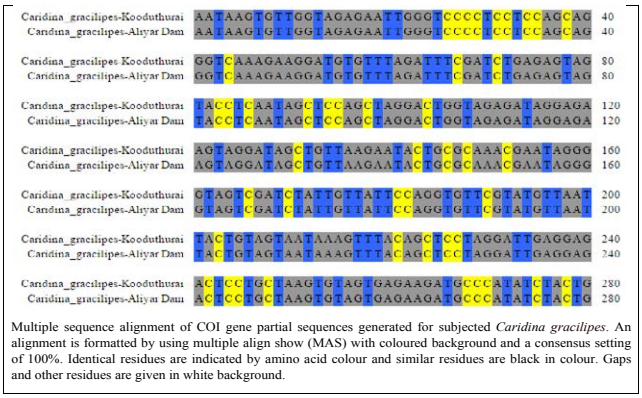
The nucleotide divergence between subjected species, and between subjected and retrieved species are depicted in Table 2. In the subjected category, the mean divergent rate of different *Macrobrachium* species was 3.628 witha maximum of 15.363 (between *M. malcolmsonii* and *M. lamarrei*) and minimum of 0.701 (between *M. lamarrei* and *M. lamarrei lamarroids*). The divergence of *Caridina* species within themselves was 2.697. When *Macrobrachium* and *Caridina* species were combined in one category, the mean divergence value was 6.934 with a maximum of 23.268 and minimum of 1.026. *M. lamarrei* alone showed 3.132% of mean divergence value within themselves taken from three different locations with a maximum of 5.370 and minimum of 1.707. Therefore, between two genera, *Macrobrachium* and *Caridina* the divergence was >3.0% in most of the cases. However, in few cases the divergence value was <3.0%, where the morphological characters play a vital role in species discrimination. In contrast to this, when the same species of different locality showed >3.0% divergence value (*M. lamarrei* of Kooduthurai vs. *M. lamarrei* of Aliyar Dam) they may be either sub-species of cryptic in nature. When retrieved species are included with subjected species >3% divergence value appeared at 12 combinations, of which 4 in within *Macrobrachium* species and 8 in *Macrobrachium* and *Caridina*, and none of the combinations within *Caridina* as well as within *M. lamarrei* showed >3.0% variation. The interspecific distance ranged from 13.2-19.9% between the species, *Macrobrachium borellii*, *Macrobrachium brasiliense*, *Macrobrachium jelskii*, *Macrobrachium*



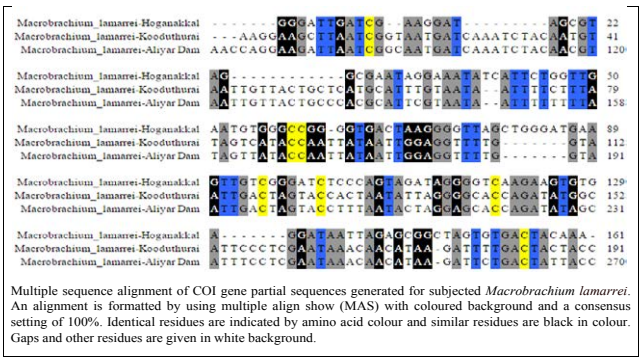
Multiple sequence alignment of COI gene partial sequences generated for subjected *Macrobrachium* species. An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.



Multiple sequence alignment of COI gene partial sequences generated for subjected *Macrobrachium* with *Caridina* species. An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.



Multiple sequence alignment of COI gene partial sequences generated for subjected *Caridina gracilipes*. An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.



Multiple sequence alignment of COI gene partial sequences generated for subjected *Macrobrachium lamarrei*. An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background.

**Plate 1:** Number of amino acid residues within *Macrobrachium* species, within *Caridina* species, between *Macrobrachium* and *Caridina* species, and within *Macrobrachium lamarrei*.

*olfersii*, *Macrobrachium crenulatum*, *Macrobrachium americanum*, *Macrobrachium carcinus* and *Macrobrachium acanthurus*, and the intraspecific distance ranged from 0-3.3% among different population of *Macrobrachium amazonicum* has been reported using COI sequences [36].

## Phylogenetic information and tree topology

The predicted phylogenetic information, such as synonymous (Ks) and non-synonymous (Ka) substitutions, transitional (Ts) and transvertional (Tv) substitutions, and saturation, index of substitutional saturation (Iss) and critical value of index of substitutional saturation (Iss.c) are presented in Table 3. In the subjected category, when *Macrobrachium* species were compared within themselves, the Ka was higher (2.181) than that of Ks (0.739), which indicates the possibility of occurrence of more deleterious mutation and less silent mutation. Similarly, the Tv was higher (0.37) than that of Ts (0.26), which indicates the fact that these sequences have more phylogenetic information. Thus, little saturation occurred in these sequences, which was confirmed by the predicted lower Iss.c value (0.790) than that of the Iss (0.916) and therefore, little phylogenetic differences existed between sequences. When more species were included by retrieving, the sequences seemed to be saturated with more phylogenetic information, because the Iss.c was higher (0.935) than that of the Iss (0.777). The phylogenetic tree topology of *Macrobrachium* species (subjected) formed one clade, in which, *M. lamarrei* was aligned alone at the base of the tree and two clusters, of which one was formed by *M. malcolmsonii* and *M. lamarrei lamarroids* as sister taxa and the another was formed by *M. lamarrei* taken from two different places as sister taxon at the top of the tree (Plate 2a). When retrieved *Macrobrachium* was included with subjected *Macrobrachium,* a polyphyletic tree was appeared with two clades and two clusters. At the base, *M. malcolmsonii* (retrieved) was formed a clade, and the next clade with *M. lamarrei* (subjected) was formed in between two clusters. The first cluster consisted of *M. lamarrei* only, of which two subjected and one retrieved. The second cluster was formed by *M. lamarrei lamarroids* (subjected) and *M. malcolmsonii* (one subjected and one retrieved) (Plate 2b).

When the species of two genera, *Macrobrachium* and *Caridina* were combined, the Ka was higher (2.178) than that of Ks (0.719), which also indicates the possibility of occurrence of more deleterious mutation than that of the silent mutation. Similarly, the Tv was also higher (0.40) than that of Ts (0.27), which indicates the fact that these sequences have still more phylogenetic information. However, saturation might have not been occurred in these sequences, because of two different genera, which was confirmed by the predicted higher Iss.c value (0.886) than that of the Iss (0.768) and more phylogenetic differences existed between sequences. When retrieved species of

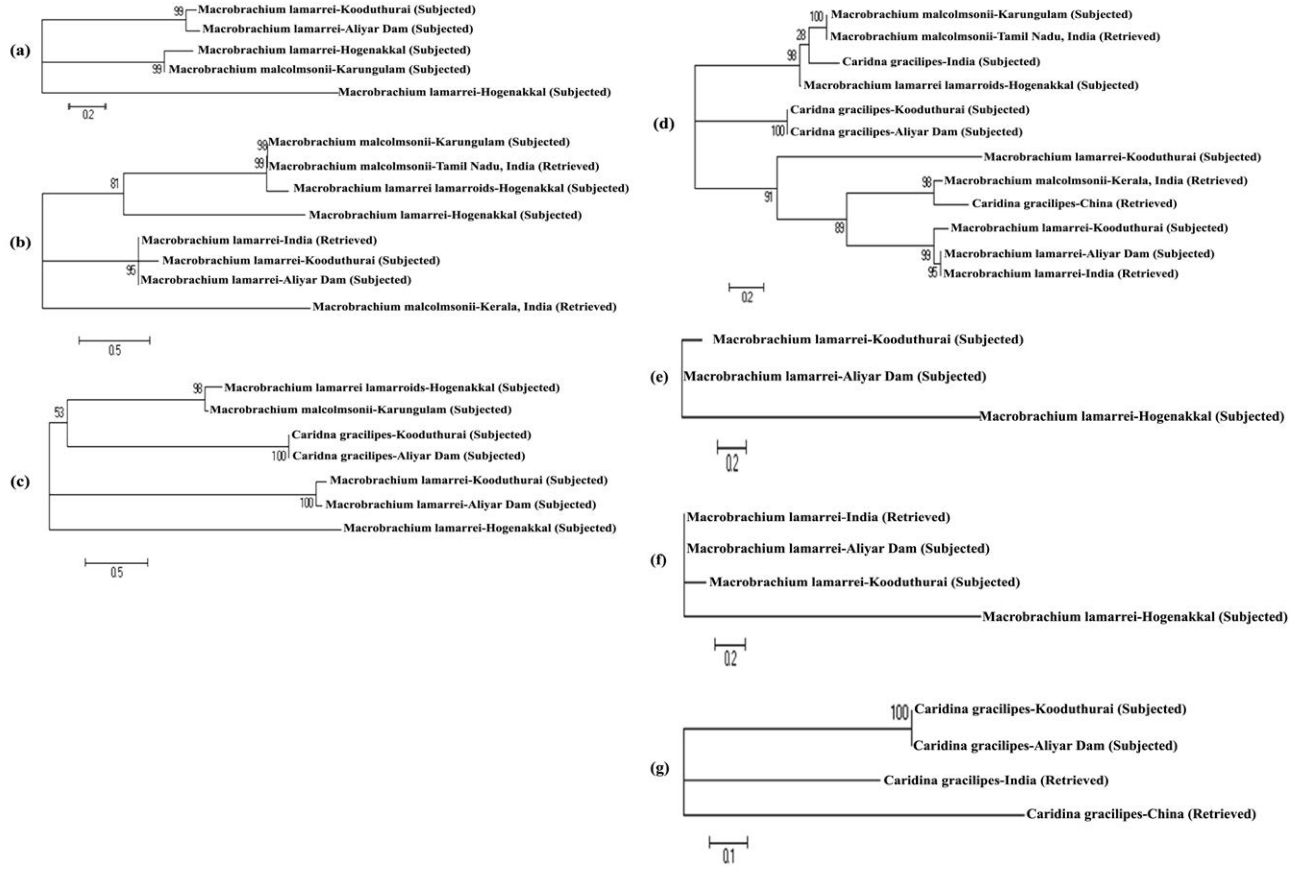



**Plate 2.** a-*Macrobrachium* species (subjected); Plate 2b-*Macrobrachium* species (subjected and retrieved); Plate 2c-*Macrobrachium* and *Caridina* species (subjected); Plate 2d-*Macrobrachium* and *Caridina* species (subjected and retrieved); Plate 2e-*Macrobrachium lamarrei* (subjected); Plate 2f-*Macrobrachium lamarrei* (subjected and retrieved); Plate 2g-*Caridina* species (subjected and retrieved).

*Macrobrachium* and *Caridina* were included with the subjected species, the sequences showed no saturation with very highest Iss.c (1.062), which indicates more phylogenetic information in this study (Table 3). Therefore, both generic and species differences existed due to genetic differences, which in turn lead to species discrimination. The phylogenetic tree topology constructed with subjected species of *Macrobrachium* and *Caridina* formed one clade with *M. lamarrei* at the base and three clusters as sister taxon each with two species. The first cluster was formed by *M. lamarrei*, the second by *C. gracilipes,* and the third by *M. malcolmsonii* and *M. lamarrei lamarroids* at the top of the tree (Plate 2c). When both subjected and retrieved species of *Macrobrachium* and *Caridina* were taken in one group four clusters and a clade were formed. The first cluster was formed by *M. lamarrei* (one retrieved and two subjected). The second cluster was formed by two retrieved species (*M. malcolmsonii* and *C. gracilipes*) as sister taxon. Then a single distinct clade with subjected species of *M. lamarrei* was formed. The third cluster was formed by the subjected *C. gracilipes*. And the forth cluster was formed by *M. lamarrei lamarroids* (subjected), *C. graclipes* (retrieved) and *M. malcolmsonii* (both retrieved and subjected) as sister taxon (Plate 2d).

In the case of *M. lamarrei* taken from three different locations, the Ka was higher (2.165) than that of Ks (0.761), which also indicates the possibility of occurrence of more deleterious mutation in the sequences. Similarly, the Tv was also higher (0.33) than that of Ts (0.23), which indicates that these sequences have phylogenetic information. Though the Iss.c was higher (0.812) than that of the Iss (0.760), little saturation seemed to occur between these sequences, because of the same species, which showed only little difference between Iss.c and Iss (0.052) (Table 3). When retrieved species of *M. lamarrei* were included the sequences also showed little saturation with more phylogenetic information (Iss.c, 805; Iss, 615) (Table 3) due to locality/ country variation. The phylogenetic tree topology of subjected *M. lamarrei* formed a separate clade and a cluster (Plate 2e). When retrieved species of *M. lamarrei* was included, it joint in the cluster at the top (Plate 2f).

The phylogenetic information for subjected *Caridina* species was not predicted because of the less sampling, but, this was calculated when the retrieved *Caridina* species were included and they also showed little saturation with some phylogenetic information (Iss.c, 806; Iss, 702) (Table 3) due to locality/ country variation. The phylogenetic tree of *C. gracilipes* (both subjected and retrieved) formed two separate clades with retrieved species and a cluster of subjected species at the top (Plate 2g).

Species that have a wide distribution, in heterogeneous or geographically isolated environments can have a phenotype variation, because they are prone to show plastic responses to different environmental influences. The presence of plastic characters in the genus *Macrobrachium* makes the accurate determination of

species more difficult and problematic [37,38]. The environment-dependent plasticity and the phenotypic variations stem from genetic or behavior differences between individuals are from ontogenetic developmental or combining of all these factors [39]. On the other hand, morphological characters may often be undergoing convergent evolution as they are under similar selective pressure [40]. The species of the genus *Macrobrachium* have high intraspecific variation and a single species may have genetic diversity and structured populations **.** A wide distribution and a great morphological variation during ontogenesis including the possibility of morphotypes within the species have been reported in congeneric species such as *Macrobrachium rosenbergii* [41], *M. amazonicum* [42], *M. grandimanus* [43-45] and *Macrobrachium olfersii* [46-49].

The development of an organism (ontogeny) expresses all the intermediate forms of its ancestors throughout evolution (phylogeny). Freshwater prawns of the genus *Macrobrachium* [50] (Crustacea: Palaemonidae) are a highly diverse group of decapod crustaceans thought to have originated from marine ancestors, some of which subsequently migrated towards fresh water in more than 1 wave; hence its members are known to inhabit the entire range of habitats from purely marine areas to inland hill streams and impounded water bodies [51-53]. In the present study, *Macrobrachium* showed > 3.0% interspecies divergence. It has been suggested that origin of *Macrobrachium* occurred in the late Oligocene or early Miocene [54]. It has been reported that species of palaemonoid, atyoid and alpheoid are not closely related [55]. Atyidae are the ancient inhabitants of freshwater, having diverged early from an ancestral marine stock [56]. Therefore, in this study, *Macrobrachium* and *Caridina* showed some distance. However, in *Caridina*, only one species, *C. gracilipes* was studied, thus, it is not possible to detect the plasticity. Moreover, it is important to mention here that very little is known on the phylogeny of Caridea due to the paucity of higher level cladistic and genetic studies [3].

## Conclusion

In this study, the sequences of *Macrobrachium* and *Caridina* showed considerable degree of variation in the amino acid sites, which indicate that they were very well discriminated. The observed > 60% base composition indicates lower abundance of NUMTs genes. The >3.0% mean divergence recorded between different species of *Macrobrachium* indicates their clear discrimination. The subjected *C. gracilipes* always sat with a separate cluster and only the retrieved *C. gracilipes* was aligned with *M. malcolmsonii* and *M. lamarrei lamarroids*, and mostly *M. lamarrei* formed a separate cluster or clade. Therefore, the species of *Macrobrachium*, and *Caridina* are clearly delineated from each other as the phylogenetic information obtained through mt-COI partial gene sequences are more conserved and less subjected to evolutionary forces, and thus, these species are genetically distinct.

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## Author Affiliations

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*Department of Zoology, Bharathiar University, Tamil Nadu, India*