

RESEARCH ARTICLE

Phylogenetic positions of some species of the genus *Macrobrachium* Bate, 1868 (Crustacea, Decapoda, Palaemonidae) in Sri Lanka

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Abstract: *Macrobrachium* species, are an economically important freshwater prawn group in Sri Lanka. These species are recognized by their local names and many synonyms can be found for one species. Identification of taxonomic positions and species boundaries within the genus is important to obtain reliable information for application in aquaculture and biodiversity conservation programmes.

Approximately 471bp partial sequences from mitochondrial 16S rRNA using seven *Macrobrachium* species were collected during the present study. Six of them were collected from the southern part of Sri Lanka and their phylogenetic positions among the relevant species that have been recorded within the region were determined. The analysis resulted in five clades, of which three showed monophyletic lineages (*M. australe* group, *M. latimanus* group, and *M. latidactylus* group). *M. malcolmsonii* is in a sister clade to *M. rosenbergii* while *M. scabriculum* joined with *M. idae*. The estimated intraspecific nucleotide divergence level varied from 0 – 6.07% while it varied from 5.21 – 10.84% at the interspecific level. The phylogenetic positions of the samples recorded within the region are discussed.

Keywords: Freshwater prawns, *Macrobrachium*, mitochondrial 16S gene region, phylogenetic relationships, Sri Lanka.

INTRODUCTION

Freshwater prawns of the genus *Macrobrachium* Bate, 1868¹ are important macro invertebrates in freshwater and estuarine systems throughout the tropical and warm temperature areas of the world. This genus can be ecologically separated into two groups. Most species are widely distributed and require specific saline concentrations to complete larval development (euryhaline species) and others are land locked species

with limited distributions and complete their entire life cycle in freshwater^{2,3}. So far more than 200 species have been described around the world and there are more to be described. *Macrobrachium* species have a high commercial value in the fisheries sector⁴. A study⁵ listed 86 *Macrobrachium* species that are economically important and among them at least 11 species have gained great commercial value in different countries. The giant freshwater prawn *M. rosenbergii*⁶ is farmed commercially, both within its natural range and outside.

Taxonomically, this genus is one of the most challenging decapod crustacean groups. The most distinguishable morphological characters of this genus are the rostrum and the second pereopod, which are highly variable among species⁷. Most of the studies on *Macrobrachium* species are based on morphology^{2,3,5,7,8} and in recent years few studies have been published using molecular data. Some previous studies⁹⁻¹² have produced important clarifications for Australian *Macrobrachium* species based on 16S mitochondrial gene region. Further, based on the same gene region another study³ suggested multiple origins of *Macrobrachium* species, region wise. Subsequently, two researchers^{14,15} carried out phylogenetic studies using multiple gene regions. For these studies data were collected from a wide geographical range mainly from the south and south-eastern Asian regions. However, Sri Lankan *Macrobrachium* samples were not included. Collectively, the above studies produced interesting information and insight regarding *Macrobrachium* taxonomy.

In Sri Lanka, *Macrobrachium* species are an economically important group. Many species are

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recognized by their local names and many synonyms can be found for one species, which makes studies more complicated. Therefore, identification of species boundaries within this genus is important to obtain information for applications in aquaculture and biodiversity conservation.

Two detailed taxonomic studies are recorded from Sri Lankan *Macrobrachium* species. One study¹⁶ described six species from different geographic locations and the other⁸ described twelve species collected from all over the country. However, these recorded species are only briefly described and their morphological characters are not properly illustrated using figures. To date no taxonomic studies at molecular level have been conducted with this genus in Sri Lanka. Therefore, the objective of the present study was to collect partial sequences of mitochondrial 16S ribosomal gene region from seven *Macrobrachium* species collected mainly from the southern part of Sri Lanka to determine their phylogenetic positions among the relevant species that have been recorded within the region.

METHODS AND MATERIALS

Seven *Macrobrachium* species were identified based on morphological features⁴ and used in this study. Among them 6 species (*M. rosenbergii*, *M. scabriculum*¹⁷, *M. idea*¹⁷, *M. australe*¹⁸, *M. latimanus*¹⁹ and *M. latidactylus*²⁰) were collected from the southern part of Sri Lanka mainly from the streams and small rivers of 2 major river basins: Nilwala and Walawe. *M. malcolmsonii*²¹ was collected from the Gal Oya river system in the eastern part of Sri Lanka (Figure 1). Three to four specimens from each species were used to extract DNA. Reference samples were stored at the Department of Zoology, University of Ruhuna, Matara for further studies. Sampling localities are given in Table 1.

DNA was extracted using the easy DNA extraction kit (QIAGEN, USA). A fragment of the 16S rRNA mitochondrial gene was amplified by Polymerase Chain Reaction (PCR) using primers 1471- 5'CCTGTTTANCAAAAACAT3' and 1472-

Table 1: Sampling localities and gene bank accession numbers for sequences

| Species (abbrev) | Location | Sample code | Genbank accession numbers |
|-------------------------------------|--------------------------|-------------|---------------------------|
| <i>M. rosenbergii</i> (M. rose) | Sri Lanka ¹ | SriA | FJ595480* |
| | Sri Lanka ^{1,2} | SriB | FJ595481* |
| | India | Ind | DQ004836 |
| | Thailand | Tha | AY203908 |
| | Papua New Guinea | Pap | AY203906 |
| <i>M. malcolmsonii</i> (M.male) | Australia | Aus | AY203918 |
| | Sri Lanka ³ | Sri | GU987055* |
| <i>M. scabriculum</i> (M.scab) | India | Ind | AY730050 |
| | Sri Lanka ¹ | Sri | GU987059* |
| <i>M. idea</i> (M.idae) | India | Ind | AY730055 |
| | Sri Lanka ^{1,2} | Sri | GU987058* |
| | Taiwan | Twn | DQ194930 |
| <i>M. australe</i> (M.aust) | Australia | Aus | AY282777 |
| | Sri Lanka ¹ | Sri | GU987057* |
| | Philippine | phi | DQ194905 |
| | Taiwan | Twn | DQ194904 |
| <i>M. latimanus</i> (M.lati) | New Guinea | New | DQ681290 |
| | Sri Lanka ^{1,2} | Sri | GU987056* |
| | Japan | Jap | DQ194938 |
| | Taiwan | Twm | DQ194936 |
| <i>M. latidactylus</i> (M.latid) | Philippine | Phi | DQ194937 |
| | Sri Lanka ¹ | Sri | GU987060* |
| | Taiwan | Twn | EU493140 |
| | Thailand | Tha | DQ194946 |
| | Philippine | phi | DQ194945 |
| | China | Cha | DQ194943 |
| <i>Palaemon</i> (Palmon) | Malaysia | Mal | DQ194944 |
| | | | FM986647 |

* Sequences derived from this study

Sampling localities: 1-Nilwala river basin, 2- Walawe river basin, 3- Gal Oya basin (refer to Figure 1)

Table 2: Percentage of sequence divergence (uncorrected 'p' distance) among populations and out group for the 16S gene region. Abbreviations are given in Table 1.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | | |
|-----------------|-------|-------|-------|-------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|---|
| 1 M.rose,SriA | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 M.rose,SriB | 0.22 | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 M.rose,Ind | 0.00 | 0.22 | - | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 M.rose,Tha | 0.00 | 0.22 | 0.00 | - | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 M.rose,Pap | 5.86 | 6.07 | 5.86 | 5.86 | - | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 M.rose,Aus | 5.21 | 5.42 | 5.21 | 5.21 | 0.65 | - | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 M.malc,Sri | 6.07 | 6.29 | 6.07 | 6.07 | 5.42 | 5.21 | 0.00 | - | | | | | | | | | | | | | | | | | | | | | | |
| 8 M.malc,Ind | 6.07 | 6.29 | 6.07 | 6.07 | 5.42 | 5.21 | 0.00 | - | | | | | | | | | | | | | | | | | | | | | | |
| 9 M.scab,Sri | 9.58 | 9.79 | 9.58 | 9.58 | 10.22 | 9.79 | 8.06 | 8.06 | - | | | | | | | | | | | | | | | | | | | | | |
| 10 M.scab,Ind | 9.12 | 9.33 | 9.12 | 9.12 | 9.99 | 9.55 | 7.82 | 7.82 | 0.43 | - | | | | | | | | | | | | | | | | | | | | |
| 11 M.idae,Sri | 7.59 | 7.82 | 7.59 | 7.59 | 8.46 | 8.03 | 8.03 | 8.03 | 6.96 | 6.73 | - | | | | | | | | | | | | | | | | | | | |
| 12 M.idae,Twn | 7.59 | 7.82 | 7.59 | 7.59 | 8.46 | 8.03 | 8.03 | 8.03 | 6.96 | 6.73 | 0.00 | - | | | | | | | | | | | | | | | | | | |
| 13 M.idae,Aus | 8.03 | 8.25 | 8.03 | 8.03 | 9.11 | 8.68 | 8.46 | 8.46 | 6.75 | 6.52 | 0.86 | 0.86 | - | | | | | | | | | | | | | | | | | |
| 14 M.aust,sri | 10.21 | 9.99 | 10.21 | 10.21 | 9.99 | 10.2 | 10.2 | 10.2 | 10.9 | 10.7 | 8.90 | 8.90 | 9.77 | - | | | | | | | | | | | | | | | | |
| 15 M.aust,Phi | 9.99 | 9.78 | 9.99 | 9.99 | 9.78 | 9.56 | 10.2 | 10.2 | 10.5 | 10.2 | 8.69 | 8.69 | 9.53 | 1.52 | - | | | | | | | | | | | | | | | |
| 16 M.aust,Twn | 9.99 | 9.78 | 9.99 | 9.99 | 9.78 | 9.56 | 10.2 | 10.2 | 10.5 | 10.2 | 8.69 | 8.69 | 9.53 | 1.52 | 0.00 | - | | | | | | | | | | | | | | |
| 17 M.aus,New | 10.21 | 9.99 | 10.21 | 10.21 | 10.21 | 9.99 | 9.78 | 9.76 | 10.9 | 10.7 | 9.12 | 9.12 | 9.53 | 1.30 | 2.39 | 2.39 | - | | | | | | | | | | | | | |
| 18 M.lati,Sri | 9.33 | 9.11 | 9.33 | 9.33 | 9.33 | 9.11 | 9.54 | 9.54 | 8.72 | 8.46 | 8.88 | 8.88 | 9.32 | 8.23 | 8.48 | 8.48 | 8.05 | - | | | | | | | | | | | | |
| 19 M.lati,Jap | 10.41 | 10.19 | 10.41 | 10.41 | 10.41 | 8.68 | 8.46 | 8.68 | 7.63 | 7.82 | 8.45 | 8.45 | 8.88 | 8.05 | 8.26 | 8.26 | 7.83 | 2.82 | - | | | | | | | | | | | |
| 20 M.lati,Twn | 10.63 | 10.41 | 10.63 | 10.63 | 8.89 | 8.68 | 8.89 | 8.89 | 7.84 | 8.03 | 8.67 | 8.67 | 9.10 | 8.26 | 8.48 | 8.48 | 8.05 | 3.04 | 0.22 | - | | | | | | | | | | |
| 21 M.lati,Phi | 10.41 | 10.19 | 10.41 | 10.41 | 8.68 | 8.46 | 8.68 | 8.68 | 7.63 | 7.82 | 8.45 | 8.45 | 8.88 | 8.05 | 8.26 | 8.26 | 7.83 | 2.82 | 0.00 | 0.22 | - | | | | | | | | | |
| 22 M.lati,d,Sri | 10.41 | 10.19 | 10.41 | 10.41 | 10.19 | 10.2 | 8.89 | 8.89 | 8.47 | 8.23 | 9.10 | 9.10 | 9.54 | 8.91 | 9.13 | 9.13 | 8.26 | 7.59 | 7.38 | 7.59 | 7.38 | - | | | | | | | | |
| 23 M.lati,d,Twn | 10.84 | 10.62 | 10.84 | 10.84 | 9.54 | 9.75 | 8.89 | 8.89 | 8.63 | 8.44 | 9.54 | 9.54 | 9.97 | 9.78 | 9.35 | 9.35 | 9.34 | 7.81 | 7.59 | 7.81 | 7.59 | 1.95 | - | | | | | | | |
| 24 M.lati,d,Tha | 10.41 | 10.19 | 10.41 | 10.41 | 9.93 | 9.98 | 8.89 | 8.89 | 8.47 | 8.23 | 8.67 | 8.67 | 9.11 | 8.69 | 8.92 | 8.92 | 8.04 | 7.59 | 7.38 | 7.59 | 7.38 | 1.29 | 2.38 | - | | | | | | |
| 25 M.lati,d,Phi | 10.19 | 9.97 | 10.19 | 10.19 | 9.75 | 9.98 | 9.12 | 9.11 | 8.91 | 8.66 | 9.32 | 9.32 | 9.76 | 9.13 | 9.35 | 9.35 | 8.69 | 7.59 | 7.38 | 7.59 | 7.38 | 1.73 | 0.65 | 2.17 | - | | | | | |
| 26 M.lati,d,Cha | 10.62 | 10.41 | 10.62 | 10.62 | 9.32 | 9.54 | 9.12 | 9.11 | 8.9 | 8.66 | 9.32 | 9.32 | 9.76 | 9.56 | 9.35 | 9.35 | 9.35 | 8.69 | 7.59 | 7.38 | 7.59 | 1.73 | 0.21 | 2.17 | 0.43 | - | | | | |
| 27 M.lati,d,Mal | 10.41 | 10.19 | 10.41 | 10.41 | 9.54 | 9.75 | 8.89 | 8.89 | 8.69 | 8.44 | 9.10 | 9.10 | 9.54 | 9.35 | 9.57 | 9.57 | 8.91 | 7.38 | 7.16 | 7.38 | 7.16 | 1.52 | 0.43 | 1.95 | 0.22 | - | | | | |
| 28 Palmon | 20.32 | 20.54 | 20.32 | 20.32 | 19.84 | 19.8 | 19 | 19 | 18.7 | 18.7 | 17 | 16.9 | 17.2 | 19 | 19.2 | 19.2 | 18.1 | 16.6 | 17.4 | 17.7 | 17.4 | 16.4 | 16.6 | 16.6 | 16.6 | 16.6 | 16.6 | 16.4 | 16.38 | - |

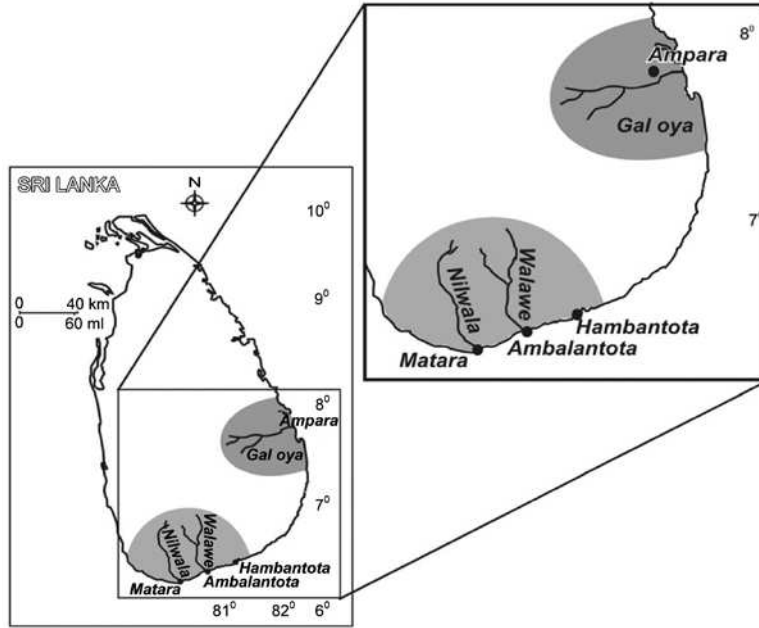


Figure 1: Sampling localities of the current study

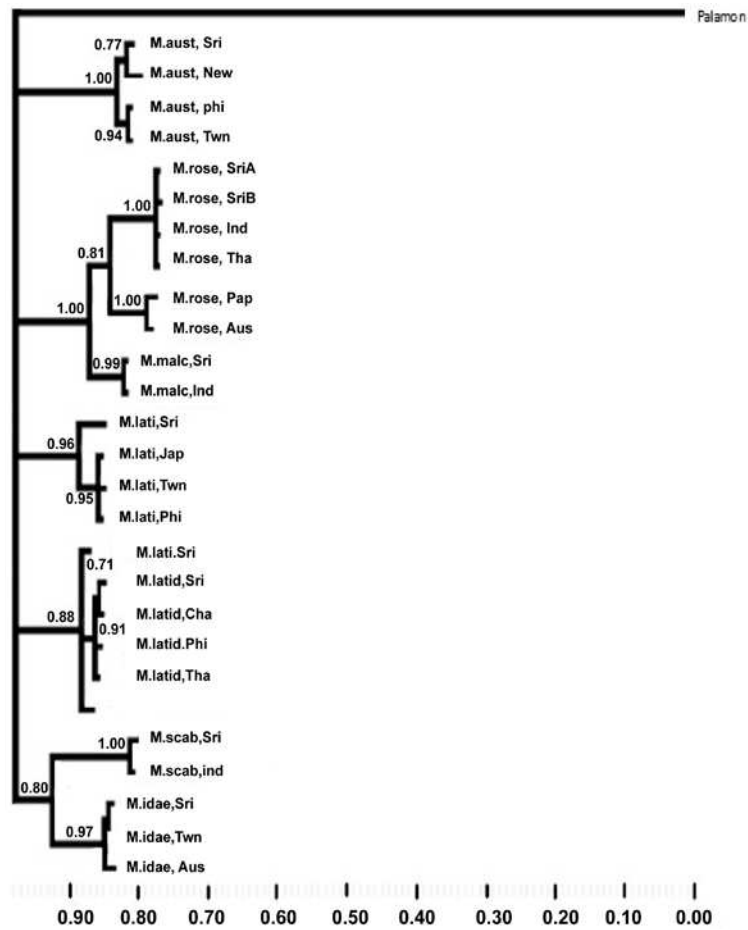


Figure 2: Majority-rule consensus tree derived from Bayesian analysis. Numbers indicate posterior probability values (> 0.7) that supported for each relationship. Refer Table 1 for abbreviations.

5'AGATAGAAACCAACCTGG3' described in a previous study²² which were initially designed for freshwater crayfish. Double stranded PCR products were obtained in a total reaction volume of 25 μ L, containing 5 μ L of 10X PCR buffer, 0.4 mM of each dNTP, 0.8 μ M of each primer, 4 mM MgCl₂, 1 unit of Taq polymerase and 2 μ L of DNA extract. PCR amplification was carried out using the following temperature regime: an initial denaturation step of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, an annealing temperature of 50°C for 30 s and an extension of 72°C for 30 s. This was followed by an additional extension of 72°C for 3 min. PCR products were purified using a QIAGEN QIAquick PCR purification kit, with a final elution volume of 50 μ L per individual. The quality of the PCR products was visualized on 1.5% agarose gels. Sequencing was carried out in both directions using the same primer pairs for PCR. Sequences were obtained using the Big Dye Terminator version 3.1 protocol (Applied Biosystems, USA) and analyzed using an ABI 3130xl Genetic Analyzer (with KB base caller; Applied Biosystems, USA).

Sequence chromatograms were viewed and edited manually using a combination of Edit View and SeqPup programmes²³. Once edited, multiple alignments were performed using Clustal X programme²⁴ with multiple alignment parameters of gap penalty equal to 10–15 and gap extension penalty equal to 3–5. Positions of uncertain alignment were excluded to produce a stable data set. Sequences were then imported into PAUP4.0b10 programme²⁵ for phylogenetic analysis. Additional sequences for the recorded species within the region and out group species²⁶ were collected from Genebank (Table 1) and used to justify the phylogenetic positions of Sri Lankan species among them. Sequences derived for this study were deposited in Genebank (Accession number GU987055 - GU987060 and FJ595480 - FJ595481) (Table 1).

Bayesian analyses (BA) were performed with MrBayes version 3²⁷ using the model selected by MrModeltest programme²⁸. Markov chain Monte Carlo (MCMC) chains were run for 1x10⁶ generations, and trees were saved each 100 generations (with the 1st 1000 trees being discarded as 'burn in'). The probability values greater than 95% were considered as significant support for relationships. The neighbour-joining analyses [Minimum Evolution option (ME)] were performed using distance calculated under the same model of evolution as the Bayesian analysis. Maximum Parsimony (MP) analyses were performed with gaps treated as missing data and heuristic search option was used with tree bisection-reconnection (TBR) branch swapping and 100 stepwise random additions. Bootstrapping was performed with

1000 replicates for all analyses. The level of support for each analysis is indicated on clads of the phylogenetic tree.

RESULTS

Sequences of approximately 471bp in length were obtained from 16S rRNA gene. The mean nucleotide composition was A= 36.58% , T= 30.57%, C= 22.57%, G=10.28%. This indicates that the 16S rRNA region of the mtDNA is the adenosine and thymine rich in the palaemonids. On the basis of the ModelTest , the GTR+I+G model of sequence evolution was chosen and the parameters specified by this model [(A-C)=1.1231, (A-G) 20.5595, (A-T)=2.0745, (C-G)=0.0001, (C-T)=9.5918, (G-T)=1.0000 and gamma distribution parameter=0.2301] were used for further analysis. Pairwise distances (uncorrected 'p' distance) for the data set are given in Table 2.

In this study, the estimated intraspecific nucleotide divergence level varied from 0 – 6.07% while it varied from 5.21 – 10.84% for the interspecific level. All three methods of phylogenetic analysis (BA, MP, ME) produced identical tree topologies. Five clades were derived: *M. australe* group, *M. malcolmsonii* + *M. rosenbergii* group, *M. latimanus* group, *M. latidactylus* group and *M. scabriculum* + *M. idae* group. However, the generated dendrogram did not resolve the deeper level phylogenetic relationships (Figure 2).

DISCUSSION

This study was based on mitochondrial 16S rRNA gene region of seven *Macrobrachium* species collected from different geographical regions. The analysis resulted in five clades and four of them produced monophyletic lineages. *M. malcolmsonii* made a sister clade to *M. rosenbergii* while *M. scabriculum* joined with *M. idae* (Figure 2).

Congeneric crustacean species commonly exhibit significant differences at the 16S rRNA mtDNA gene ranging from 2 – 17% sequence divergence²⁹⁻³². This divergence range is also supported by other recent studies conducted on *Macrobrachium* species^{9-11,14}. Therefore, the divergence levels that became evident in this study between and among species are typical for those observed between crustacean species (Table 2).

M. australe showed the lowest intraspecific nucleotide divergence that varied from 0 – 2.4%. *M. australe* group is defined with high support and

contains two clades. The southeast Asian samples were grouped together while Sri Lankan *M. australe* is grouped with New Guinean sample with lower support (0.77). The nucleotide divergence level between Sri Lankan and New Guinean sample was 1.3% while the Sri Lankan sample varied from the others with 1.5% divergence level.

M. malcolmsonii formed a sister clade which was basal to *M. rosenbergii*. The nucleotide divergence level between the two species varied from 5.2 – 6.4%. Two haplotypes were found within Sri Lankan *M. rosenbergii*. The *M. rosenbergii* samples collected from the South and South-East Asia were grouped together to accept the hypothesis of eastern and western division of this species along the Huxley's line suggested by a previous study³³. The nucleotide sequence divergence between the two clades varied from 5.2 – 6%. This value was quite similar to the divergence level found between *M. rosenbergii* and *M. malcolmsonii* samples. *M. malcolmsonii* is so far reported from the South Asian region and it is worthwhile to conduct further phylogenetic studies using more gene regions to reveal their relationship.

In *M. latimanus* group, the Sri Lankan sample was positioned basal to the other samples. This separation of Sri Lankan *M. latimanus* from other samples (nucleotide divergence level 3%) was greatly supported by high bootstrap values. This result was also supported by a previous study¹⁴, which reported up to 3.2% intra specific sequence divergence levels for *Macrobrachium* species. Within the *M. latidactylus* group, Sri Lankan and Thailand samples have given basal support to the clade. The Sri Lankan sample differed from the inner group by 1.5 – 2.0% nucleotide divergence level while this level was 1.2 – 2.2% for the Thailand sample. *M. idae* made a sister clade to *M. scabriculum* with lower support. Within the *M. idae* clade, two Asian samples were similar to each other and showed 0.9% nucleotide divergence level from the Australian sample. *M. scabriculum* is so far reported from South Asian region and the Sri Lankan sample differs from the Indian sample by 0.4% nucleotide divergence level.

Detailed morphological study of Sri Lankan *Macrobrachium* taxonomy has been conducted previously⁸. In this study, many species have been collected from outside the southern Sri Lanka. From the twelve species described in the above study, the present study has found six species in the southern part of Sri Lanka. However, the previous studies have not highlighted the phylogenetic relationships of the species. The current study is the first molecular based study of the genus *Macrobrachium* in Sri Lanka. More taxon

sampling with additional gene regions are required to better understand the phylogenetic relationships among Sri Lankan samples.

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References

1. Bate C.S. (1868). On a new genus, with four new species of freshwater prawns. *Proceedings of the Zoological Society of London* **1868**: 363-368.
2. Holthuis L.B. (1950). The decapod of the Siboga expedition. part x. the Palaemonidae collected by the Siboga and Snellius expeditions, with remarks on other species, part I: sub family Palaemoninae. *Siboga-Expedition Leiden* **39**: 1-268.
3. Johnson D.S. (1973). Notes on some species of the genus *Macrobrachium* (Crustacea: Decapoda: Caridae: Palaemonidae). *Journal of the Singapore National Academy of Science* **3**: 273-291.
4. Holthuis L.B. (1980). *FAO Fisheries Synopsis No 125*, FAO Species Catalogue. vol. 01-shrimps and prawns of the world: an annotated catalogue of species of interest to fisheries. Food and Agriculture Organization of the United Nations, Rome.
5. Jayachandran K.V. (2001). *Palaemonid Prawns: Biodiversity, Taxonomy, Biology and Management*. Science Publishers Inc., Enfield, USA.
6. De Man J.G. (1879). On some species of the genus *Palaemon* Fabar with descriptions of two new forms. *Notes from the Royal Zoological Museum of the Netherlands at Leyden* **1**: 165-184.
7. Short J.W. (2004). A revision of Australian river prawn, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). *Hydrobiologia* **525**: 1-100.
8. Costa H.H. (1979). The Palaemonidae of the inland waters of Sri Lanka. *Ceylon Journal of Science (Biological Sciences)* **13**: 39-64.
9. Murphy N.P. & Austin C.M. (2003). Molecular taxonomy and phylogenetics of some species of Australian palaemonid shrimps. *Journal of Crustacean Biology* **23**: 169-177.
10. Murphy N.P. & Austin C.M. (2004a). Multiple origins of the endemic Australian *Macrobrachium* (Decapoda: Palaemonidae) based on 16S rRNA mitochondrial

- sequences. *Australian Journal of Zoology* **52**: 549–559.
11. Murphy N.P. & Austin C.M. (2004b). Phylogeography of the widespread Australian freshwater prawn, *Macrobrachium australiense* (Decapoda, Palaemonidae). *Journal of Biogeography* **31**: 1065–1072.
 12. Murphy N.P., Short J.W. & Austin C.M. (2004). Re-examination of the taxonomy of the *Macrobrachium australiense* Holthuis (Decapoda: Palaemonidae) species-complex: molecular evidence for a single species. *Invertebrate Systematics* **18**: 227–232.
 13. Murphy N.P. & Austin C.M. (2005). Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. *Zoological Scripta* **34**: 187–197.
 14. Liu M.Y., Cai Y.X. & Tzeng C.S. (2007). Molecular systematics of the freshwater prawn genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) inferred from mtDNA sequences, with emphasis on East Asian species. *Zoological Studies* **46**: 272–289.
 15. Wowor D., Muthu V., Meier R., Balke M., Cai Y. & Ng P.K.L. (2009). Evolution of life history traits in Asian freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis. *Molecular Phylogenetics and Evolution* **52**: 340–350.
 16. Mendis A.S. & Fernando C.H. (1962). A guide to the freshwater fauna of Ceylon. *Bulletin of the Fisheries Research Station, Ceylon* **12**:1–160.
 17. Heller C. (1862). Neue crustaceen gesammelt während der weltturnseglung der k.k.Fregatte Novara; zweiter voläufiger bericht. *Verhandlungen des Kaiserlich-Königlichen Zoologisch-botanischen Gesellschaft in Wien* **12**:519–528.
 18. Guérin-Mèneville F.E. (1838). Crustaces, arachnids et insectes. In: *Voyage Autour du Monde, Excuté par Order du Roi, Sur la Corvette de Sa Majesté, La Coquille, Pendant les Annes 1822,1823, 1924 et 1825*, (Ed. L.J. Duperrey). Zoologie 2 (no.2, sect.1): 1–47 (Crustaces), 48–319 (Arachnides et Insectes), Pls 1–5 (Crustaces), 1–21(Insectes). Arthus Bertrand, Paris.
 19. Von Martens E. (1868) Ueber einige ostasiatische Susswasserthiere. *Archiv für Naturgeschichte* **34**(1): 1–67.
 20. Thallwitz J. (1891). Ueber einige neue indo-pacifische Crustaceen (vorläufige mittheilung). *Zoologischer Anzeiger* **14**: 96–103.
 21. Edwards H.M. (1844) Crustaces. Voyage dans l'Inde, par Victor Jacquemont, pendant les années 1828a 1832. *Description des Collections* **4**:1–9.
 22. Crandall K.A., Lawler S.H. & Austin C.M. (1995). A preliminary examination of the molecular phylogenetic relationships of some crayfish genera from Australia (Decapoda: Parastacidae). *Freshwater Crayfish* **10**: 18–30.
 23. Gilbert D.G. (1997). *SeqPup Software*. Indiana University, USA.
 24. Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research* **24**: 4876–4882.
 25. Swofford D.L. (2000). PAUP*. Phylogenetic Analysis Using Parsimony (and other methods). Version 4. Sinauer Associates, Inc. Publishers, Massachusetts, USA.
 26. Weber F. (1795). *Nomenclator Entomologicus Secundem Entomologiam Systematicam ill. Fabricii Adjectis Speciebus Recens Detectis et Varietatibus*. pp. vii+171. Chilonii et Hamburgi, Germany.
 27. Ronquist F. & Huelsenbeck J.P. (2003). MrBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
 28. Nylander J.A.A. (2004). *MrModeltest Version 2.2*. Evolutionary Biology Centre, Uppsala University, Sweden.
 29. Sarver S.K., Silberman J.D. & Wals P.J. (1998). Mitochondrial DNA sequence evidence supporting the recognition of two subspecies or species of the Florida Spiny Lobster. *Journal of Crustacean Biology* **18**: 177–186.
 30. Crandall K.A., Fetzner J.W. Jr, Lawler S.H., Kinnersley M. & Austin C.M. (1999). Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfishes (Decapoda: Parastacidae). *Australian Journal of Zoology* **47**: 199–214.
 31. Jarman S.N., Nicol S., Elliott N.G. & McMinn A. (2000). 28S rDNA evolution on the Eumalacostraca and the phylogenetic position of Krill. *Molecular Phylogenetics and Evolution* **17**: 26–36.
 32. Fetzner J.W. Jr & Crandall K.A. (2001). Genetic variation. In: *Biology of Freshwater Crayfish* (Ed. D.M. Holdich). pp. 291–326, Blackwell Science, Oxford.
 33. de Bruyn M., Wilson J.A. & Mather P. (2004). Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant fresh water prawn, *Macrobrachium rosenbergii*. *Molecular Phylogenetics and Evolution* **30**:(1)251–257.