Proceedings of the Sixth Academic Sessions, University of Ruhuna 2009
Vol. 6 pgs 26 - 31

Phylogenetic position of Sri Lankan freshwater prawn *Macrobrachium rosenbergii* (Decapoda: Palaemonidae)

D. H. N. Munasinghe
Department of Zoology, University of Ruhuna
✉ *dhnm@zoo.ruh.ac.lk*

Abstract
The giant freshwater prawn, *Macrobrachium rosenbergii* is the largest known palaemonid in the world. It is one of the most economically important freshwater crustaceans around the world including Sri Lanka. Its natural range extends from south to southeast Asia including Papua New guinea, Australia and around pacific islands. The availability of two geographically distinct groups has been revealed in past studies and is suggested to recognize and re-name eastern and western groups as *M. rosenbergii* and *M. dacqueti,* respectively. The present study examined the phylogenetic position of Sri Lankan *M. rosenbergii* using mitochondrial 16S rRNA partial sequences. Sequences from 13 geographically separated populations were used in this analysis. Results supported the division of two clades indicating geographically separated genetically different western and eastern groups. Between two clades the nucleotide divergence level ranged from 4.89% - 5.96%. The degree of nucleotide divergence within western clade ranged from 0.00% to-0.64% and within eastern clade it varied from 0.21%-0.85%. Presence of two haplotypes was indicated (nucleotide divergence level 0.21%) within Sri Lankan populations and they grouped within the western clade. Between two haplotypes found, haplotype II is common among six other populations. This evidence suggests that further studies are needed to confirm whether giant freshwater prawn in Sri Lanka is different from hototype of *M. rosenbergii* and could be treated as the recently designated lectotype, *M. dacqueti.*

Keywords: *Macrobrachium rosenbergii*, freshwater prawn, phylogeny, Sri Lanka, 16S mitochondrial DNA

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* is the largest known palaemonid in the world. It can be found in natural water bodies of south and southeast Asia including Papua New guinea and Australia and around pacific islands (Marther and Brayn, 2003). This is economically most important within the genus and widely fished where it is found and cultured almost throughout natural distribution and beyond. In the last decade, average *M. rosenbergii* production rose by some 10- 48% in quantity and 20-24.5% in value (FAO 2007). In Sri Lanka, *Macrobrachium rosenbergii* has gained a value as an important organism for aquaculture. Therefore, to increase the production in 1970's, National Aquaculture Development Authority of Sri Lanka has launched a free stocking program of *M. rosenbergii* in selected medium irrigation tanks.

However, the taxonomic recognition of *M. rosernbergi* is still unstable. So far, the name M. *rosenbergii* has 15 associated synonyms and there have been some debates to know whether it is just one species. According to *www.sealifebase.org* (Palomares and Pauly, 2009), *M. rosenbergii* is the valid and accepted name. Holthuis (1950) recognized it as only one wide ranging species and Jonson (1960) recommended that there are two subspecies of *M. rosenbergii*: the western and the eastern. This hypothesis was further supported by morphological and allozyme data (Hedgecock *et al.* 1979, Lindenfelser 1984,) as well as molecular work (de Bruyn *et al.*, 2004a; Chand *et al.*, 2005). These molecular studies showed a sharp division between the western and eastern populations. Bruyn *et al.* (2004a) suggested that Huxley's line could be considered as the best biological barrier to separate these two subspecies. The most recent study reported on *M. rosenbergii* is by Wower and Ng (2007) based on adult morphological characters of this species. They easily recognized the availability of two species and suggested to name eastern species as *M. rosenbergii* and western species as *M. dacqueti.*

The distribution of *Macrobrachium* sp. in Sri Lanka was reported by Costa (1979) and Mendis and

Fernando (1962). However, only two systematic studies were carried out on Sri Lankan *Macrobrachium* species (Lindenfelser 1984; Wower and Ng 2007) with limited sampling. In Lindenfelser's (1984) study *M. rosenbergii* populations were grouped into three groups; eastern, mid-western and far western. Sri Lankan *M. rosenbergii* of Sri Lanka was grouped with far western group. Wower and Ng (2007) defined only two groups: eastern and western and Sri Lankan sample grouped with western group. Bruyn *et al*'s (2004a) study was conducted using molecular sequence data, but this was mainly targeted on Southeast Asian populations., The current study is therefore important because this study expands the data range of the *M. rosenbergii* populations by adding data from south Asian populations and it helps to confirm the phylogenetic position of Sri Lankan *M. rosenbergii* species within the region.

Thus, the aim of this study is to investigate phylogenetic position of Sri Lankan *M. rosenbergii* using data from mitochondrial 16S rRNA partial sequences and to discuss the utility of the results in aquaculture and conservation programs in Sri Lanka.

Methodology

Sample collection

Three to ten *Macrobrachium rosenbergii* samples were analyzed from four locations and details are given in the Table 1. DNA was extracted using DNeasy extraction kit (QIAGEN company). PCR amplifications of partial 16S rRNA mitochondrial gene were carried out using universal 16S mtDNA primers 1471 and 1472 (Crandall et al 1995). Total genomic DNA was used as template and the following reaction concentrations were used in total volume of 25µl: 5µl of 10X PCR buffer, 0.4mM of each dNTP, 0.8µM of each primer, 4mM $MgCl_2$, 1 unit of Taq polymerase and 2µl of DNA extract. Thermal cycling was followed by an initial denaturation step of 95° for 5 min, 30 cycles of 95°C for 30 seconds, an annealing temperature of 50°C for 30 seconds and an extension of 72°C for 30 seconds and final extension temperature 72°C for 3 minutes. PCR products were gel visualized and purified using QIAquick PCR purification kit (QIAGEN company). Sequencing reactions were carried out using Big Dye Terminator ver 3.1 protocol (Applied Biosystems) and analyzed using an ABI 3130xl Genetic analyzer (with KB base caller; Applied Biosystems, Foster City, CA, USA). Sequencing reactions were carried out for both directions. Nineteen sequences representing 13 geographically different regions were used to complete the data. *M. malcomsonii* and *M. lar* were used as out groups. Sequences derived from this study were deposited in the Genbank and additional sequences were obtained from the Genbank (Table 2).

Table 1. Sample collecting localities, the number of samples examined and number of haplotypes identified from each population.

Place	Number of samples	Number of haplotypes identified
Negambo	10	One
Matara	10	Two
Ambalanthota	3	One
Tangalle	3	Two

Data analyses

Sequence chromatograms were viewed and edited manually using of Edit View program. The multiple alignments of the data set were performed using Clustal X (Thompson et al 1997) with default settings (gap penalty= 15, gap extension penalty= 6.66 and DNA transition weight= 0.50). Sequences were then imported into PAUP 4.0b10 (Swofford 2000) for phylogenetic analysis. The model of evolution was selected by Mr Modeltest program (Nylander 2004). Neighbour-Joining (NJ) analysis was performed using the resulted model of evolution. Maximum Parsimony (MP) analysis was performed with gaps treated as missing data and heuristic search option was used with general search option. Bootstrapping was performed with 1000 replicates for both NJ and MP analysis. The same model of evolution was used to perform Bayesian analyses (BI) with MRBAYES version 3 (Ronquist and Huelsenbeck 2003). Markov chain Monte Carlo (MCMC) chains were run for $1X10^6$ 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 level of support (bootstrap and posterior probability support) for each analysis is indicated on clads of the phylogenetic tree. Pairwise distances (uncorrected 'P' distance) for the data set are given in the Table 3.

Results

Approximately 470 bp of the mitochondrial 16s rRNA gene region was obtained for the analysis. Two haplotypes were identified from four locations studied from Sri Lanka (Genbank accession Numbers- FJ595480 and FJ595481).

Parsimony and Baysian analyses showed identical tree topologies and both supported only up to the separation of two geographically distinct groups and did not resolve deeper level phylogenetic relationships (Figure 1).

Table 2. Sequence accession numbers in the Genbank, their geographical location and sample codes used in this study.

Accession number	Sample locality	Sample code
AY203918[1]	QLD, Australia	AU1
AY203921[1]	NT, Australia	AU2
AY203916[1]	WA, Australia	AU3
DQ647674[2]	China	CHI
DQ642882[2]	Hong Kong	HOK
DQ004836[2]	India	IND
AY203909[1]	Irian Jaya, Indonesia	ID2
AY203913[1]	Java, Indonesia	ID1
AY203912[1]	Malaysia	M1
AY203915[1]	Malaysia	M2
AY203906[1]	Papua New Guinea	PAP
AY203910[1]	Philippines	PHI
FJ595480*	Sri Lanka	SR1
FJ595481*	Sri Lanka	SR2
DQ194959[2]	Taiwan	TAW
AY203908[1]	Thailand	TH1
AY203911[1]	Thailand	TH2
AY203914[1]	Vietnam	VI1
AY203907[1]	Vietnam	VI2
AY203922[1]		
AY730050[3]		↑*M. lar*
		↑*M. malcomsonii*

Note: [1]de Bruyn *et al.*, [2]Liu *et al.*, [3] Parhi *et al.*, *current study

However NJ analysis resolved deeper level relationships up to some extent (Figure 2). *Macrobrachium rosenbergii* populations clustered into two distinct groups with 4.89% - 5.96% nucleotide divergence level, representing geographically distinct groups as revealed in previous studies (Lindenfelser 1984; de Bruyn *et al.* 2004a; Chand *et al.* 2005; Wower and Ng 2007). This division received high bootstrap and posterior probability support from two methods of analysis. Australian sample came from Northern Territory and sample from China appears to be the ancestral to eastern and western clade respectively. However, the results did not resolve separate branch for Sri Lankan and Indian samples within the western clade as resulted in Lindenfelser's (1984) study. The degree of nucleotide divergence within western clade ranged from 0.00% to-0.64% and within eastern clade it varied from 0.21% - 0.85% (Table 3). The two haplotypes identified from Sri Lanka varied from 0.21% nucleotide divergence level. Among these two, haplotype II is common among other six populations.

Discussion:

The nucleotide divergence levels observed between two clades are consistent with the divergence levels exhibited by other crustacean groups (Sarver *et al.*, 1998; Jarman *et al.*, 2000; Schubart *et al.*, 2000; Fetzner and Crandal 2001). Therefore, the suggestion of dividing this species into two is supported by divergence levels (4.8%-5.9%) observed in this study. According to de Brayns' (2004a) study, Huxley's line is the major geographical barrier that prevented the gene flow between two regions for this species. This hypothesis is also common to the other biological taxa which Huxley (1868) modified the historical bio-geographical barrier from Wallace's line to Huxley's line based on zoological data.

Two haplotypes were identified from twenty-six samples analysed in Sri Lanka. The haplotype II is common among six other populations. This may be due to recent gene flow among these populations or translocation of this species for aquaculture purposes. *M. rosenbergii* is commercially important and currently there is a trend to expand *M. rosenbergii* culture in Sri Lanka. If the presence of haplotype II is as a result of translocations, this point should be taken into account with cautions. Therefore, the identification of genetic variability among populations is an important to avoid unnecessary detrimental effects that could effect on aquaculture programs.

The evidence from the present study suggests that further studies are needed to confirm whether

giant freshwater prawn in Sri Lanka is different from hototype of *M. rosenbergii* and could be treated as the recently designated lectotype, *M. dacqueti.* The Sri Lankan *M. rosenbergii* belongs to the western group thus it needs to recognize as '*M. dacqeti*'. The recognition of Sri Lankan *M. rosrnbergii* as '*M. dacqueti*' will make

Table 3. Pairwise distance ('P' distance) for the sequences analyzed in this study. Sequence codes are corresponding with the Table 2.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	MA1	-																				
2	MA2	0.43	-																			
3	VI1	0.21	0.64	-																		
4	VI2	0.00	0.43	0.21	-																	
5	TA1	0.21	0.64	0.43	0.21	-																
6	TA2	0.00	0.43	0.21	0.00	0.21	-															
7	ID1	0.21	0.21	0.43	0.21	0.43	0.21	-														
8	TA1	0.00	0.43	0.21	0.00	0.21	0.00	0.21	-													
9	CHI	0.43	0.85	0.64	0.43	0.64	0.43	0.64	0.43	-												
10	HOK	0.00	0.43	0.21	0.00	0.21	0.00	0.21	0.00	0.43	-											
11	IND	0.00	0.43	0.21	0.00	0.21	0.00	0.21	0.00	0.43	0.00	-										
12	SR1	0.21	0.64	0.43	0.21	0.43	0.21	0.43	0.21	0.64	0.21	0.21	-									
13	SR2	0.00	0.43	0.21	0.00	0.21	0.00	0.21	0.00	0.43	0.00	0.00	0.21	-								
14	PHI	5.53	5.53	5.34	5.53	5.75	5.53	5.32	5.53	5.53	5.53	5.53	5.75	5.53	-							
15	PAP	5.75	5.75	5.53	5.75	5.96	5.75	5.53	5.75	5.75	5.75	5.75	5.96	5.75	0.21	-						
16	ID2	5.75	5.75	5.53	5.75	5.96	5.75	5.53	5.75	5.75	5.75	5.75	5.96	5.75	0.21	0.43	-					
17	AU1	5.53	5.53	5.34	5.53	5.75	5.53	5.32	5.53	5.53	5.53	5.53	5.75	5.53	0.21	0.43	0.43	-				
18	AU2	5.11	5.11	4.89	5.11	5.32	5.11	4.89	5.11	5.11	5.11	5.11	5.32	5.11	0.43	0.64	0.64	0.43	-			
19	AU3	5.75	5.75	5.53	5.75	5.96	5.75	5.53	5.75	5.75	5.75	5.75	5.96	5.75	0.64	0.85	0.85	0.64	0.64	-		
20	*M.lar*	10.21	10.64	10.21	10.21	10.43	10.21	10.43	10.21	10.21	10.21	10.21	10.00	10.21	10.85	11.06	11.06	10.85	10.85	11.06	-	
21	*M.mal*	5.96	5.96	5.75	5.96	6.17	5.96	5.75	5.96	5.53	5.96	5.96	6.17	5.96	5.11	5.32	5.32	5.11	5.11	5.32	0.82	-

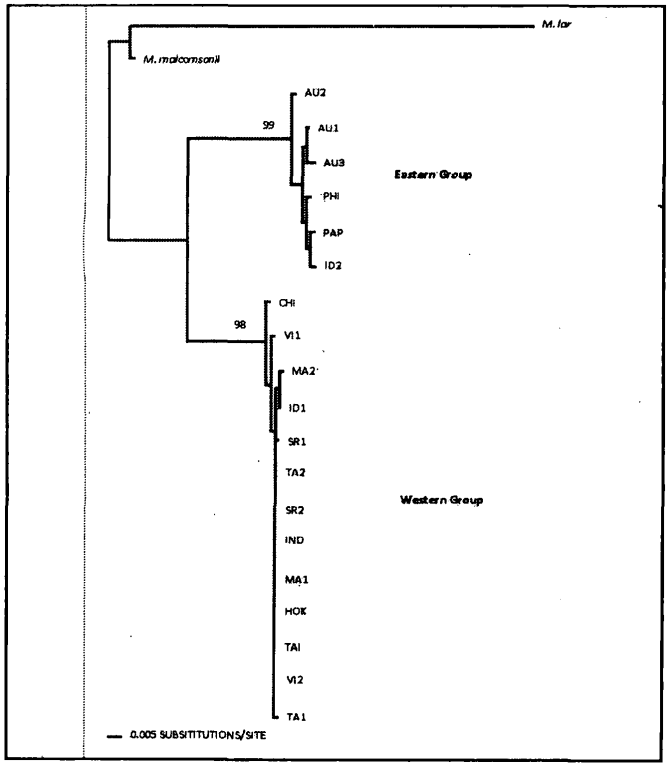

Figure 1. Phylogenetic tree derived from Neighbor-joining analysis for *M. rosenbergii* populations. Numbers indicates the bootstrap support (>90).

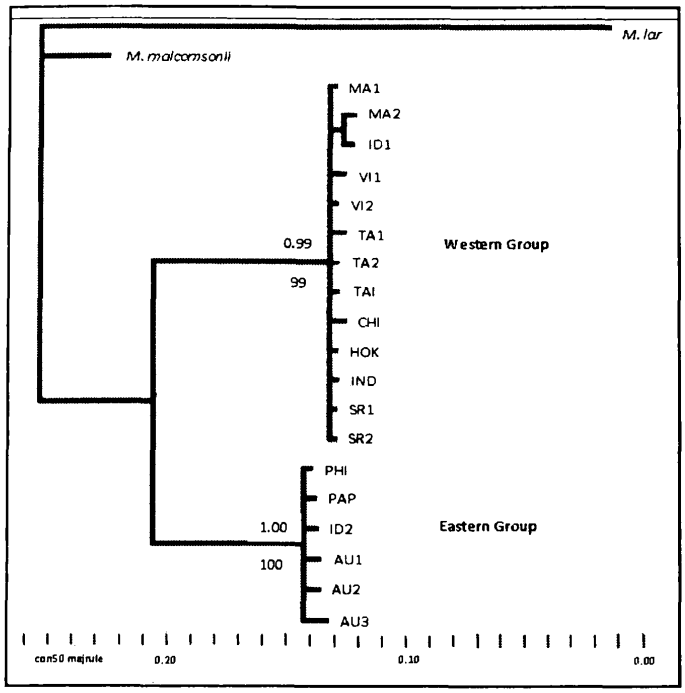

Figure 2. Tree derived from Bayesian analysis for *M. rosenbergii* populations. Numbers above the branches indicate the support of Bayesian analysis (< 0.9) and the numbers below the branches indicate the bootstrap values for Maximum Parsimony analysis (>90)

So far, basic studies are mainly based on common species of *M. rosenbergii* (Barki *et al.*, 1991, Rao 1965, Ling 1962). Thus, in future it would be another challenge to gather relevant basic information on this 'new species' to achieve better results in this industry. Wowor and Ng (2008) have proposed that although the recent taxonomic appraisals show that two distinct species can be identified, the lectotype of *M. dacqueti* be designated as neotype of *M. rosenbergii*. They recommended retaining the name of *M. rosenbergii* for commercially important species allowing subsequent use of *M. dacqueti* as its junior synonym. Wowor and Ng (2008) also proposed a new name, Macrobrachium wallacei for the current holotype of *M. rosenbergii*. However, the presence of two haplotypes within limited samples indicates the availability of genetic variability among Sri Lankan *M. rosenbergii* populations.

Conclusion

The analysis of partial sequences from mitochondrial 16S gene region indicated that Sri Lankan *M. rosenbergii* belongs to the western group, which needs to be recognized as *M. daquiti* in future. However, the point that Wowor and Ng (2008) is an important aspect to be considered seriously in this regard. Among four locations of samples studied, two types of haplotypes were identified. Haplotype I is rare among studied populations and haplotype II is common which could be seen among other six populations analyzed in this study. This indicates the availability of high genetic variability among populations and thus more population genetic studies are needed to carry out with more comprehensive sampling and using more rapidly evolving gene regions. These findings are important in planning aquaculture and environmental management programs in Sri Lanka.

Acknowledgements

My sincere gratitude should goes to Prof. C.M. Austin for providing me facilities to conduct this research at ATRF, Charles Darwin University, Australia. I am grateful to Endeavor fellowship program, which gave me an opportunity to visit Australia. I also thank to Dr. M. Shultz, Dr. S. Smith, Mr. C.M. De Silva and Ms. S. Niranjala for their kind assistance given to me while I am conducting this study. I appreciate the assistance given by the staff of the Wild Life Conservation Department while exporting samples for this study. Finally I thank two anonymous reviewers for their comments that improved the quality of this manuscript.

References

Barki, A., Karplus, I., & Goren, M. (1991). The agonistic behaviour of the three male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). *Behaviour* **116**: 252-277.

Chand, V., de Bruyn, M., & Marther, P.B. (2005). Microsatellite loci in the eastern form of the giant freshwater prawn (*Macrobrachium rosenbergii*). *Molecular Ecology notes* **5**: 308-310.

Costa H.H. (1979). The Palaemonidae of the inland waters of Sri Lanka. Ceylon *Journal of Science (Bio science)* **13**: 39-64.

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.

De Bruyn, M., Wilson, J.A., & Marther, P.B. (2004a). Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn *Macrobrachium rosenbergii*. *Molecular Phylogenetics and Evolution* **30**: 251-257.

De Bruyn, M., Wilson, J.C., & Marther, P. (2004b). Reconciling geography and genealogy: Phylogeography of giant freshwater prawns from the Lake Carpentaria region. *Molecular Ecology* **13**, 3515 – 3526.

FAO (2007). FAO year book, Fishery Statistics: aquaculture production 2005. *www.fao.org/figis*. Accessed 12 May 2007.

Fetzner, Jr J.W., & Crandall, K.A. (2001). Genetic Variation. In 'Biology of Freshwater Crayfish (Ed DM Holdich) pp 291-326. (Blackwell Science: Oxford).

Hedgecock, D., Stelmach, D.J., Nelson, K., Lindenfelser, M.E., & Malecha, S.R. (1979). Genetic divergence and biogeography of natural populations of *Macrobrachium rosenbergii*. *Proceedings of the world Mariculture Society* **10**: 873-879.

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.

Huxley, T.H. (1868). On the classification and distribution of the Alectoromorphae and Heteromorphae. *Proceedings of Zoological Society London*. 294-319.

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.

Johnson, D.S. (1960). Sub-specific and intra specific variation in some freshwater prawns of the Indo-pacific region. In : Purchon, RD (ed), Proceedings of the Centenary and Bicentenary Congress of Biology, Singapore, 1958, 259-267.

Lindenfelser, M.E. (1984). Morphometric and allozymic congruence: evolution in the prawn *Macrobrachium rosenbergii* (Decapoda: Plamonidae). *Systematic Zoology* **33**:195-204.

Ling, S.W. (1962). Studies on the rearing of larvae and juveniles and culturing of adults of *Macrobrachium rosenbergii* (de Man). *FAO Indo-Pacific Fisheries Council* **9**: 55-60.

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.

Marther, P.B, & de Bruyn, M. (2003). Genetic diversity in wild stocks of the giant freshwater prawn (*Macrobrachium rosenbergii*): Implications for aquaculture and conservation. *NAGA, World Fish Centre Quarterly* **26**: 4-7.

Mendis A.S. & Fernando C.H. (1962). A guide to the freshwater fauna of Ceylon. Bulletin of the Fisheries Statrion Ceylon 12. In: Sri Lanka freshwater fauna and fisheries published by C.H. Fernando and collaborators from 1956-2002 pp 1-160.

Nylander, J.A.A. (2004). Mr Modeltest v2.2. Evolutionary Biology Centre, Uppsala University.

Palomares, M.L.D., & Pauly, D. Eds. 2009. SeaLifeBase. World Wide Web electronic publication. www.sealifebase.org, version (01/2009).

Parhi J, Lakra, W. S., Majumdar, K.C., Mukherjee, S.C., Goswami, M., Ravinder, K., & Sahoo L. (2008). Molecular phylogeny of freshwater prawn (Genus: Macrobrachium) species based on mtDND 16S rRNN gene sequencing. *Indian Journal of Animal Sciences* **78**: 425-429.

Pethiyagoda, R. (2005). Exploring Sri Lanka's Biodiversity. *The Raffles Bulletin of Zoology Supplement*, **12**: 1-4.

Rao, R.M. (1965). Breeding behaviour in *Macrobrachium rosenbergii* (de Man). *Fish Technology India* **2**, 19-25.

Ronquist, F., & Huelsenbeck, J.P.(2003). MRBAYES. Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

Sarver, S.K., Silberman, J.D., & Walsh, 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.

Schubart, C.D., Neigal, J.E., & Felder, D.L. (2000). Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. In 'Crustacean Issue 12, The Biodiversity Crisis and Crustacea' (ed FR Schram).

Swoffaord, D.L. (2000). PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associations.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., & Higgens, 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.

Wower, D., & Ng, P.K.L. (2008). *Palaemon rosenbergii* De Man, 1879 (currently *Macrobrachium rosenbergii*; Crustacea, Decapoda): Proposed conservation of usage by designation of a neotype., *Bulletin of Zoological Nomenclature* **65**: 3428.

Wower, D., Ng, P.K.L. (2007). The giant freshwater prawn of the *Macrobrachium rosenbergii* species group (Crustacea: Caridea: Palmonidae). *The Raffles Bulletin of Zoology* **55**: 321-336.