Population genetic structure analysis of *Penaeus monodon* (Fabricius, 1798) in Sri Lanka

Final Report

Grant Number: RG/2010/BT/02

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Matara

Contents: **Page**

Section 1 - Information regarding project/Project personnel 1

Section 2 - Executive summary of the project...2

Section 3 - Report in detail...3

Section 4 - Impact of research results...15

Section 5 – Miscellaneous...17

Section 6 - Summary statement of expenditure 18

Section 7 - Signatures and recommendations..19

Annexes - 20

Final Report

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1. Information regarding Project/Project Personnel:

i) Grant Number: **RG/2010/BT/02**

ii) Title of the Project: **Population genetic structure analysis of *Penaeus monodon* (Fabricius, 1798) in Sri Lanka.**

iii) Principal Investigator: (Prof/Dr/Mr/Ms): **Dr. D.H.N. Munasinghe**

iv) Co-Investigators: - None

v) Institute where Research is being carried out: **University of Ruhuna, Matara and Genetech Pvt. Ltd, Colombo 8, Brigham Young University, Utah, USA.**

vi) Date of award/date Project was initiated: **08/ 09/ 2010**

vii) Date of completion of Project: **28/02/2014**

viii) Total allocation of funds (Rs): **1062352/=**

ix) Total spent (Rs):**1061498/=**

x) Number of Research Students employed: **N/A**

xi) Post graduate degree completed with dates: **N/A**

xii) Number of Technical Assistants and/or laborers employed and period of service: **One Technical assistant and one laborer**

xiii) Publications/Communications arising from the project during the reporting period:

Two abstracts presented at national and international conferences

One full paper submitted to a journal and pending for the decision

Section 2

Executive Summary of the Project:

Genetic variations of three *Penaeus monodon* populations in Sri Lanka were estimated using both mitochondrial and nuclear gene regions. At the initial stage, population genetic analyses were carried out using Single Stranded Conformation Polymorphism (SSCP) method using partially amplified DNA fragment (600 bp) of mitochondrial control (mtC) gene region. However, due to difficulties experienced, finally population genetic analyses were carried out using direct sequencing technique of the same gene region. Sixty three individuals that represent three populations (southern, eastern and western) were sequenced and subjected to analyses. Total of twenty five haplotypes were identified from three populations and only one haplotype was common between southern and eastern populations. Mean haplotype diversity (h) and nucleotide diversity (π) among three populations were ranged from 0.35 (Southern) to 0.92 (eastern) and from 0.5% (southern) to 2.5% (western) respectively. Analysis of molecular variance (AMOVA) indicated within population variation as 78.89% and among population variation as 21.11% indicating high intra-population diversity. The overall F_{st} value indicated significant genetic structure among three populations ($F_{st} = 0.2111$, $P = 0.00 < 0.05$). When compare results of the present study with published data, it indicates availability of genetically more structured populations in south Indian Ocean than Indo – Pacific region.

Phylogenetic status of the derived haplotypes from three populations was estimated using mitochondrial control gene region and nuclear elongation factor gene region. Phylogenetic tree derived from mtC gene region revealed that among the haplotypes recorded from other geographic locations, southern Sri Lankan haplotypes formed a genetically distinct clade, which was also confirmed by the results of partial amplification of elongation factor gene region. Results of both gene regions revealed that Sri Lankan populations are genetically more closely related with the western Pacific Ocean populations than western Indian Ocean populations. Utility of the findings of the current study in future scientific advancement, fisheries and conservation programs are discussed.

Section 3

Report in detail: should contain the following (not less than 2000 words excluding Tables and Figures)

(i) Introduction/background

Freshwater aquaculture has expanded rapidly in the Asia-pacific region over the last 30 years. The black tiger shrimp *P. monodon* is one of the most important marine crustacean species and is subjected to intense fishery exploitation and aquaculture practices worldwide (Hulata, 2001). Exploitation of shrimp fisheries may deplete the wild stocks to an unsustainable level. Moreover, the tiger shrimp aquaculture industry still relies on wild-caught brood stocks to seed farmed shrimp populations. Therefore, the origin of brood stocks and their genetic composition are significant issues that require attention. Due to the inbreeding depression effects, release of hatchery stocks with limited genetic variation may be detrimental to the viability of natural populations (Ferguson, 1995). Thus, Wild stocks can provide an immediate resource for addressing genetic diversity problems in cultured stocks, and consequently require conservation. To this end, the patterns and extent of genetic diversity that are present in wild stocks need to be adequately documented so that we can identify which stocks may carry unique genetic attributes and prioritize conservation efforts.

In Sri Lanka, early 90's was the period where the prawn farming came to the climax stage. In late 90's outburst of white spot disease made a considerable damaged to the shrimp culturing industry which earn considerable amount of foreign exchange to the county (Wijegoonawardena and Siriwardena, 1996). As indicated, major reasons for this outbreak are pollution and the maintenance of the hatcheries in a very low standard in terms of the quality (Jory, 2000), it is important to be highlighted that lack of genetic diversity within the stock may be another important issue to spread this disease rapidly among prawn farms.

To date, the population genetic data of *P. monodon* have been based on several markers such as, allozymes (Klinbunga et al., 1998; 1999; Sugama et al., 2002), randomly amplified polymorphic DNA (Tassanakajon et al., 1998), microsatellites (Brooker et al., 2000; Supungul et al., 2000; Xu et al., 2001; You et al., 2008), mitochondrial DNA (mtDNA) fragment length polymorphism (Benzie et al.; 2002) and sequence data (Kumar et al., 2007; You et al, 2008; Waqairatu et al., 2012) and nuclear DNA data (Duda and Palumbi 1999).
Although *P. monodon* was one of the major sources of income in aquaculture sector in Sri Lanka, reported studies on population structure of *P. monodon* in Sri Lankan waters are limited. Herath (2008) has conducted a preliminary study to investigate genetic variation of *P. monodon* in selected geographical regions using mitochondrial Cytochrome Oxidase I gene region. However, even with very low samples numbers, genetically different sequences have been identified from western and northwestern regions during this study. It is important to conduct aquaculture programs with the knowledge of genotypic background of the cultured species. Thus, it is necessary to screen the diversity of the wild populations before collecting brood stocks for culturing programs.

(ii) Scientific scope of the project (overall and specific objectives)

I) To achieve a sound understanding of genetic variability among natural/wild populations of *Penaeus monodon* in Sri Lanka.

II) Estimate phylogenetic status of Sri Lankan haplotypes among other geographically distributed genetic forms.

III) Which helps;

- To generate information for enhancement programs when artificial recruitment is required.
- To be used as information source for genetic upgrading of *P. monodon* through selective breeding improved on growth and disease resistant.
- To be used as information source for conservation programs when necessary.
- To compare genetic diversity pattern among Sri Lankan populations with other data available within the region.

(iii) Materials and methods (including statistical methods)

Sampling process

Samples were collected from three populations of *P. monodon*; southern, western and eastern regions (Figure 1) during 2010 - 2011. Approximately 100 samples were collected from each population. Following points were considered to minimize the bias that can be occurred during the sampling process in this study. Before collect samples from commercial catches, the origin of the samples (wild/cultured/imported) and the method of gear used were examined. Most of the samples selected were come from brush pile fishery which collected using scoop nets (which minimize the gear selectivity). Samples were collected with a size range which individual sizes of the samples vary from: carapace length approximately 20mm – 50mm in length. Samples were stored on iced or frozen immediately after capture and transported to Research Laboratory of the Department of Zoology, University of Ruhuna, Matara, Sri Lanka.

Collection of data

DNA Extraction

Approximately 20 – 25 samples were collected from each population for genetic analysis. DNA extraction was carried out using DNeasy Blood & Tissue (QIAGEN) extraction kit following the manufacturer's instructions.

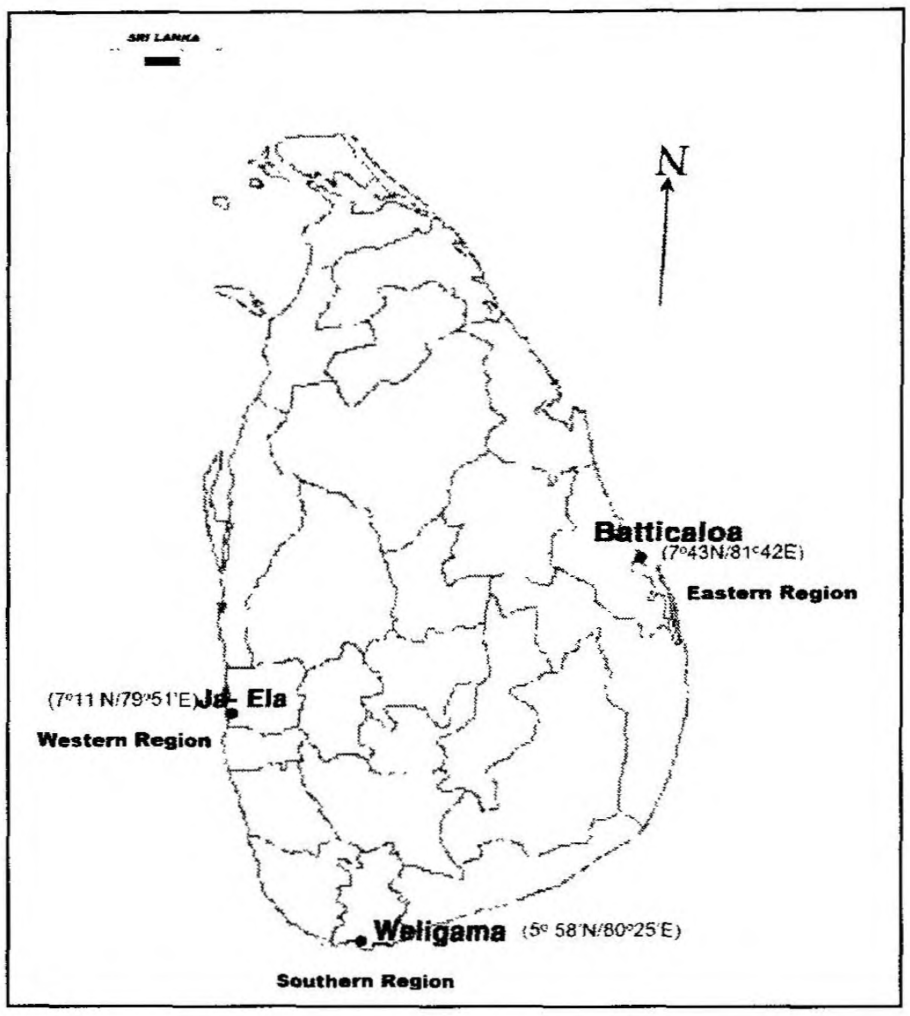

Figure 1: Sample collecting sites for the current study

Amplification of partial mitochondrial gene region:

A fragment of mitochondrial control gene region was amplified using following primers (Chu et al., 2003) : 12S (forward) 5'AAGAACCAGCTAGGATAAAACTTT 3' and 1R (reverse) 5'GATCAAAGAACATTCTTT AACTAC 3'. PCR reactions were conducted in 25µL reaction mixture contained 1× Taq polymerase buffer, 1.5mM MgC12, 0.4mM each dNTP, 0.2µm of each primer, 100ng of DNA template and 0.5U of Taq DNA polymerase. The PCR conditions used were: 2min at 94°C following 30 cycles of 30s at 94°C, 30s at 45°C, 1min at 72°C and finally, 7min at 72°C.

Analyses of Single Stranded Conformation Polymorphism Technique (SSCP) method

Successful PCR products were screened for genetic diversity using single stranded confirmation method (SSCP) (Orti et al 1997). Briefly, 10-20µl of unpurified PCR product was mixed with 5µl of loading buffer containing 0.4µl of 1M methylmercury hydroxide, 1-l of 15% ficoll loading buffer (with 0.25% bromophenol blue a nd 0.25% xylene cyanol) and 3.6-l of 1X TBE buffer (90 mM Tris, 92mM boric acid, 2.5 mM EDTA). This mixture was denetured for 4-5 minutes at 85°C and immediately chilled on ice before loading to the SSCP gel. Six to 16% polyacrylamide (39:1 acrylamide to bis acrylamide) TBE gels (16 cmX14cmX1.5mm) were run with 1XTBE buffer on verticle electrophoresis system. Refigrated water from a thermostatically controlled circulator was passed through a verticle cooling chamber to maintain the upper buffer and gel at a conatant temperature. Gels were run at constant power (8-12W) for 8-20h, and with the upper buffer temperature set to 3-15°C. Gels were stained for at least 20 min with a 2-g/ml silver nittrate solution and destained for 5 min with distilled water. Bands were also can be visualized following silver staining and were scored by comparisons of sets of individuals. However, due to practical problems, expected results were unable to achieve. Therefore, data were collected using direct sequencing technique using mitochondrial control gene region. Further, the possibility of amplify partial Elongation Factor - nuclear gene region and its utility in phylogenetic study was performed. Experiments to collect sequencing data were conducted at two places: Molecular Genetics Laboratory at Brigham Young University (BYU), USA or Molecular Unit at the University of Colombo, Sri Lanka.

Partial amplification of nuclear gene region – elongation factor was performed using primers EF3 5'GACAAGGCCCTCCGTCTTCC3' and EF4 5'GGGCACTGTTCCAATACCTCC3' (Duda and Palumbi 1999). This gene region was amplified using the same PCR reaction mixture and thermocycle parameters which were used for amplification of control gene region, except the annealing temperature which was used as 60°C. The size and quality of PCR products were assessed using 1% agarose gel electrophoresis. PCR products were sequenced using facilities available at the sequencing centre, BYU University, USA or at Macrogen Company, Korea.

Analyses of sequencing data:

Population genetic variation

Sequence alignment was performed using Bioedit V. 07 (Hall, 2004) program. Population genetic analyses were performed using ARLEQUIN (Schneider et al., 2000) program. Haplotype diversity (h) and nucleotide diversity (π) were calculated. Analysis of molecular variance (AMOVA) (Excoffier, 1992) was performed to estimate the variation contributing to the differentiations among populations and among individuals. For each level, the sum of the squared deviation, the mean squared deviation and the variance component were calculated, and the variance component was then expressed as a percentage. The significance of variance among populations was tested by 1000 random permutation test against the null hypothesis that all individuals belonged to the same population.

Phylogenetic analyses

Molecular phylogenetic analyses were performed using MEGA (V 5.2) phylogenetic analyses software and Minimum Evolution trees were constructed. Published sequences were downloaded from the Genbank to construct the data set to determine the phylogenetic status of resulted haplotypes from Sri Lankan waters.

(iv) Results/outputs

Population genetic analyses using SSCP method

For one population SSCP method produced promising results (Figure 2), however other two populations did not produced clear banding pattern for haplotypes observed.

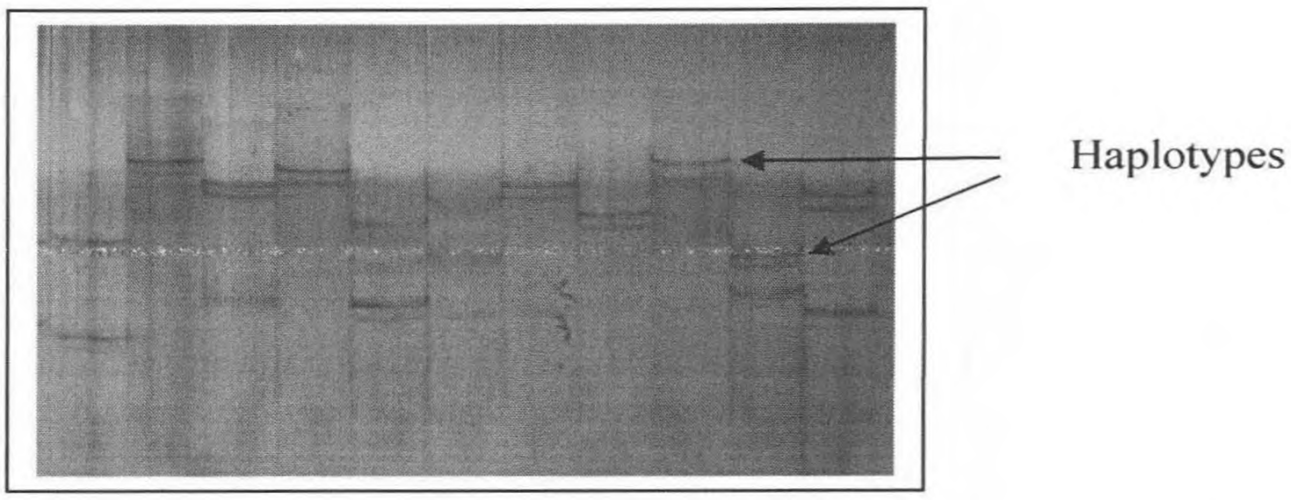

Figure 2: Gel image of SSCP analyses for western population

Population genetic analyses using direct sequencing method

Approximately 600 bp fragment of the mtDNA control region was amplified for 63 individuals of *P. monodon*. Twenty five haplotypes were resulted from three populations and sequences were deposited in the gen bank under accession numbers KF639857 - KF639882. Only one haplotype was common among southern and western populations and all other haplotypes were specific to each population. Mean haplotype diversity (h) among three populations was ranged from 0.35 (Southern) to 0.92 (Eastern). The nucleotide diversity (π) among three populations was ranged from 0.5% (Southern) to 2.5% (Western). The overall F_{st} value indicated significant genetic structure among populations ($F_{st} = 0.2111$, $P = 0.00 < 0.05$).

Phylogenetic analyses

Sequences of nuclear gene region were submitted to gene bank (pending for a number). Phylogenetic relationships among haplotypes derived from mitochondrial gene region and Phylogenetic status of haplotypes recorded around Sri Lankan waters are given in Figure 3 and 4. Results emerged for the above after analyses of nuclear gene –elongation factor are given in the Figure 5 and 6.

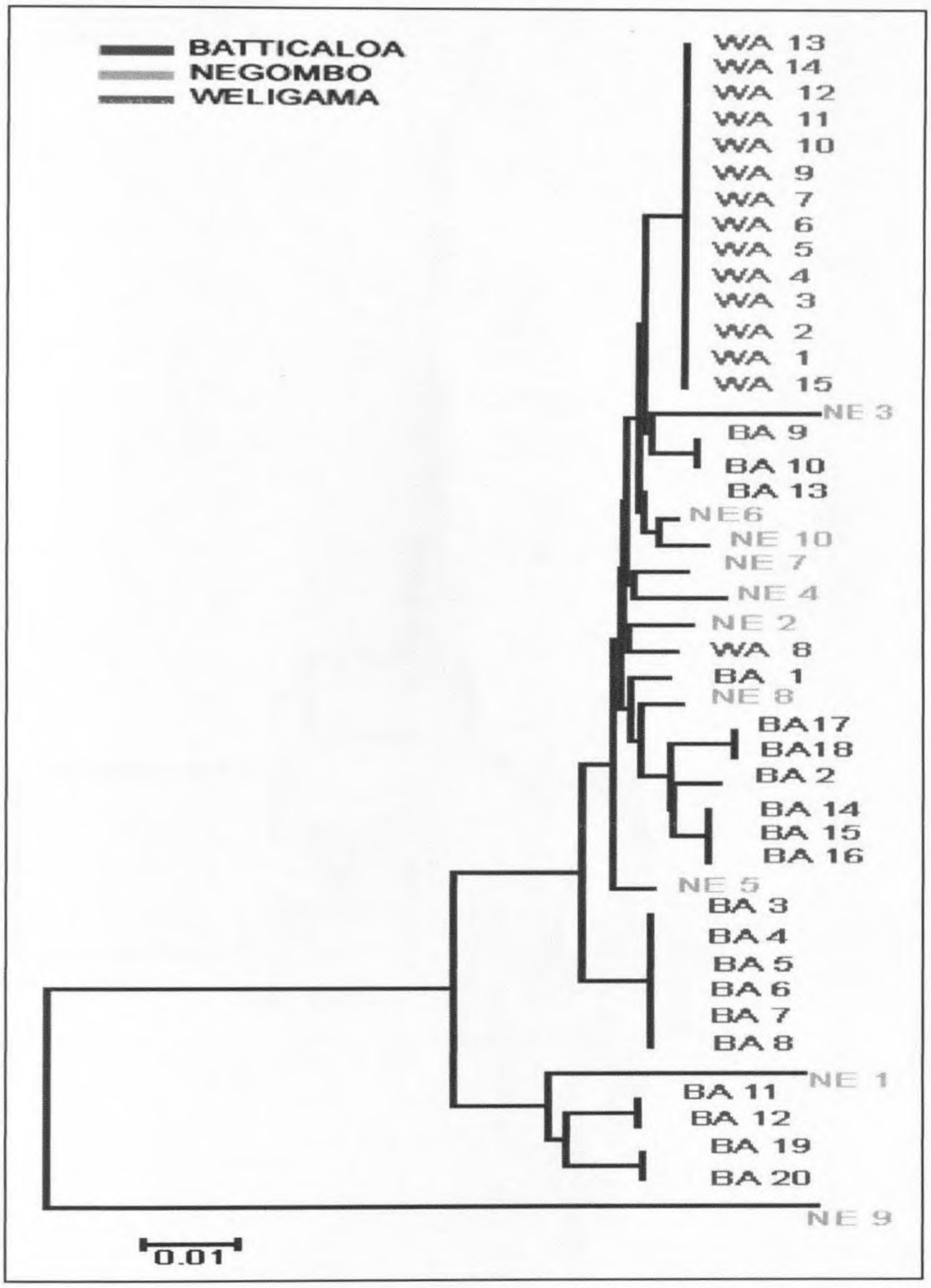

Figure 3: Phylogenetic tree (un-rooted) derived from analyses of mitochondrial control gene region to indicate relationship among haplotypes resulted from three populations

BA – Eastern Region, WA – Southern Region, NE – Western Region

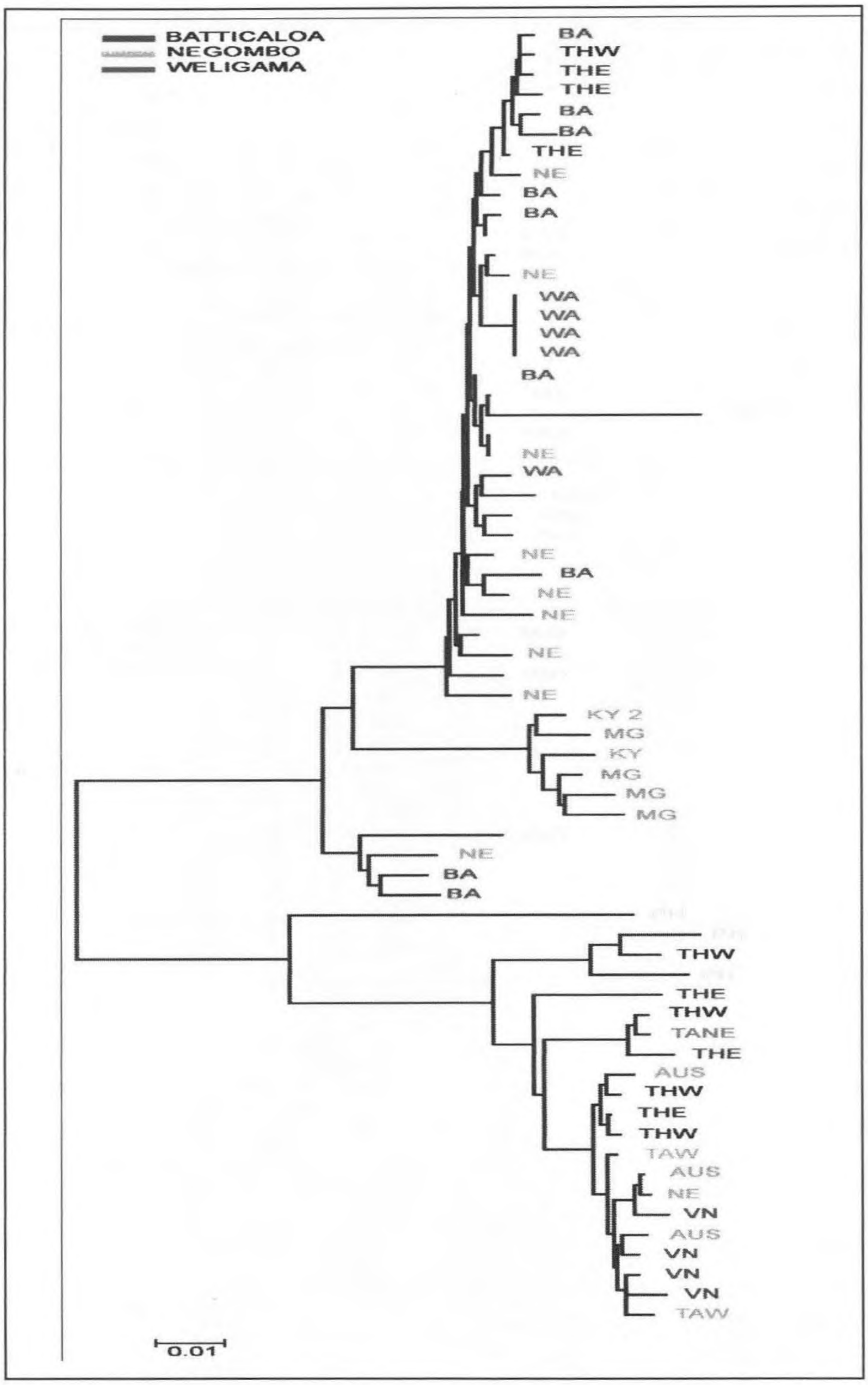

Figure 4: Phylogenetic tree (un-rooted) derived from analyses of mitochondrial control gene region to indicate relationship among haplotypes resulted from Sri Lanka and other geographical locations

Other geographical locations: AUS – Australia, IND – India, KY- Kenya, MG- Madagascar, PH-Philippine, THE - Thailand, TAW – Taiwan, TANE – Tanzania, VN – Vietnam
Sri Lankan locations: BA – Eastern Region, WA – Southern Region, NE – Western Region

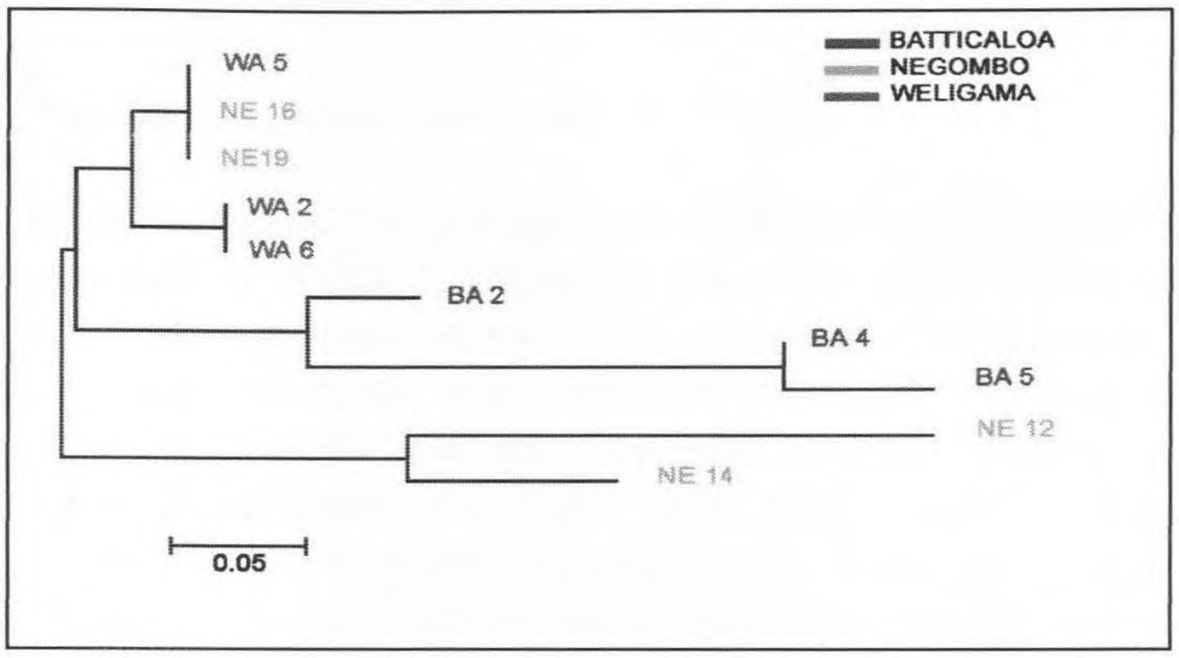

Figure 5: Phylogenetic tree (un-rooted) derived from analyses of elongation factor, nuclear gene region to indicate relationship among haplotypes resulted from three populations

BA – Eastern Region, WA – Southern Region, NE – Western Region

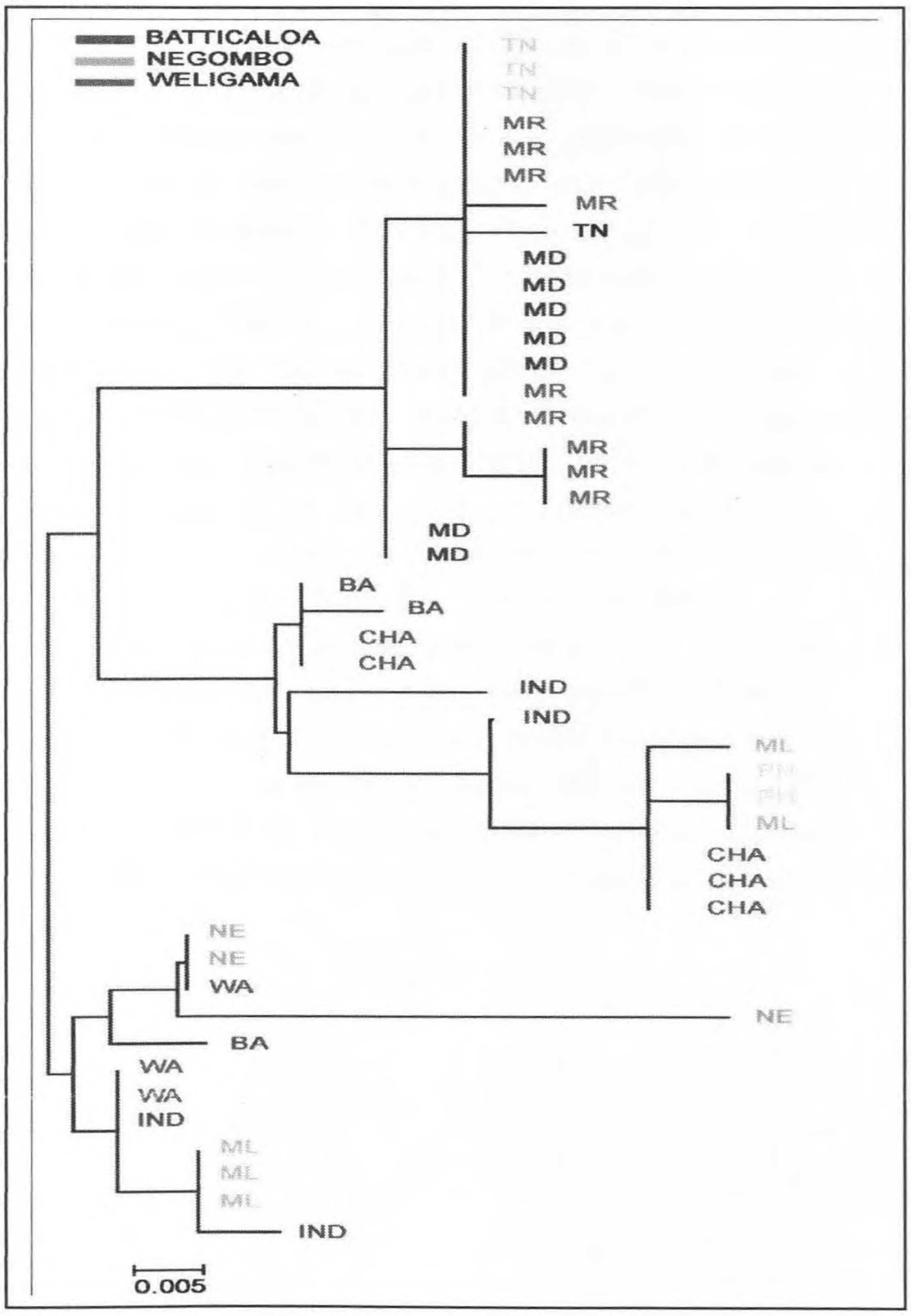

Figure 6: Phylogenetic tree (un-rooted) derived from analyses of elongation factor, nuclear gene region to indicate relationship among haplotypes resulted from Sri Lanka and other geographical locations

Other geographical locations:
CHA – China, IND – India, MD – Madagascar, MR – Mauritius, ML – Malasia, TN - Tanzania

Sri Lankan locations:
BA – Eastern Region, WA – Southern Region, NE – Western Region

(v) Discussion

Population genetic diversity

High haplotype (h) and nucleotide diversity levels (π) were observed in eastern and western populations (Table 2) indicating great genetic variation within the populations. Similar results have been reported in past studies which mitochondrial control gene region produced high haplotype diversity levels (Kumar et al., 2007; Zhou et al., 2009; You et al., 2008). In contrast to that, Southern population of the current study indicated low haplotype and nucleotide diversity levels ($h = 0.3579 +/- 0.1266$, $\pi = 0.005478 +/- 0.003295$) indicating low genetic variation within the population. These results indicate the signs of over exploitation of the population or restriction of gene flow among other populations due to isolation.

Phylogenetic status analyses

The phylogenetic genetic study on *P. monodon* in Sri Lanka was carried out using 588 bp sequence fragment of mitochondrial control gene region and nuclear elongation factor gene region. Three populations were selected from southern, western and eastern parts of Sri Lanka. High haplotype number was recorded in western and eastern populations while low haplotype number was reported from the southern population. Only one haplotype was common among southern and eastern populations and all others are specific to each population. When compared Sri Lankan haplotypes with other published *P. monodon* sequences for the relevant gene region, results indicated that Sri Lankan haplotypes are closely related with the western pacific ocean populations (Thailand, Thaiwan, Vietnam etc.) than western Indian ocean populations (Madagascar, Mauritius, Tanzania etc.). Phylogenetic tree derived from analyses of nuclear Elongation factor grouped majority of Sri Lankan samples to a separate clade with close relationship with Malaysian and Indian samples (Figure 8). This may be due to the pattern of oceanic currents which contribute to the distribution of pelagic larval stage of *P. monodon*. However, one haplotype is highly abundant in southern population which indicates low genetic variation. Occurrence of such type of low genetic variation could be possible due to over exploitation of available natural population or isolated population due to a geographical barrier available in the coastal region. This information could be utilized when selecting brood stocks for *P. monodon* culturing programs and implementation of management strategies for conservation of wild populations in Sri Lanka.

Conclusions

It has been highlighted the importance and utility of genetic information in stock identification and conservation studies for wild population. Molecular markers provide direct information to identify populations as a single unit or composed of several subunits (Chauhan and Rajiv, 2010). The results of the current study have revealed the availability of genetically diverged *P. monodon* populations in western, southern and eastern regions of Sri Lanka.

According to the results of this study, in a broad scale it can be suggested that Sri Lankan populations are genetically more closely related with the western pacific ocean populations (Thailand, Thaiwan, Vietnam etc.) than western Indian ocean populations (Madagascar, Mauritius, Tanzania etc.).

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- Professor C.V. Chandrasekaran, Molecular Unit, Department of Chemistry, University of Colombo.
- Mr. Palitha Harasgama, Technical Officer, Molecular Unit, Department of Chemistry, University of Colombo.

(vii) Problems if any, encountered during the implementation of the project

- Experienced some difficulties when conducting SSCP method.

(viii) Major findings and follow up activities.

- Genetically diverged *P. monodon* populations available in eastern, western and southern region of Sri Lanka. The results revealed that there is high genetic variation available in eastern and western populations of Sri Lankan waters while southern population shows low genetic variation.
- The results for low genetic variation could be due to either over exploitation of the population or isolation of population due to a geographical barrier. Proper management plans are need to investigate the factors responsible for this.
- Sri Lankan populations are genetically more closely related with the western Pacific Ocean populations (Thailand, Thaiwan, Vietnam etc.) than western Indian Ocean populations (Madagascar, Mauritius, Tanzania etc.), Populations with unique haplotypes such as southern population need to be highlighted for conservation.
- Routing monitoring programs are necessary to maintain population genetic structure of the wild populations which can be affected through factors such as over exploitation, pollution and climate changes.

Section 4

Impact of Research results:

i). Relevance of results achieved to scientific advancement

Black tiger prawn, *P. monodon* is one of the commercially important crustacean species all over the world and therefore, many studies have been published on the genetic variation of this species in different geographic locations. According to the past investigations, there are genetically diverge two groups in the Indian Ocean: western and easterner Indian Ocean populations (Duda and Palumbi 1999). Sri Lanka is situated in the middle of this dividing point and there is a scarcity of genetic data on this species from Sri Lanka to determine the status of the Sri Lankan *P. monodon* as well as to demarcate the border line between the two regions (Ridgway and Sampayo 2005). Those information are important for zoographic studies in the world.

Therefore, by publishing data of the current study (in genbank and publications), a contribution could be made to future scientific studies of the field.

Collection and documentation of genetic data of geographically separated populations are also important in production of new *P. monodon* varieties. Such as Genetically Improved Farmed Thilapia (GIFT), there is a possibility to produce genetically improved tiger prawn varieties. In such occasions, results of current are important to select genetically different individuals which could be utilized to conduct experiments in order to select the best variety.

ii). Relevance of results achieved to national/socio-economic development

Prawn farming is the most significant commercial scale aquaculture conducted in Sri Lanka. Export of farmed prawns contribute 9% to total fishery exports and are the largest contributor to fish exports apart from food fish (70%) (Investment proposal by Ministry of Agriculture, Animal Production and Development, Industries, Fisheries and Tourism Eastern Province - 2013). As a highly profitable industry, there is a growing demand to invest on the prawn farm industry. Thus, it is important to focus on producing healthy populations with high growth rate which can achieve high marketable values and a profit. Apply precautions to prevent disease outbreaks also an important point. Proper understanding of biological aspects is important to conduct successful culturing programs, especially, it is important to have knowledge on population genetics and population ecology. Maintaining genetic diversity of a population is a key factor to achieve above targets and which could be achieved by maintaining a genetically diverged brood stock. Therefore, basic reserch are important to identify genetically diverged wild populations.

The results of the current study have revealed the availability of genetically diverged *P. monodon* populations in western southern and eastern regions of Sri Lanka. These findings could be utilized when selecting brood stocks for culturing programs which genetically diverged individuals should be mixed up to produced more productive offspring with high growth rates and resistant to diseases. On the other hand when artificial recruitment is necessary for ongoing culturing programs, appropriate individuals could be selected from those genetically diverged populations according to available genetic information.

According to the results of this study, in a broad scale it can be suggested that Sri Lankan populations are genetically more closely related with the western pacific ocean populations (Thailand, Thaiwan, Vietnam etc.) than western Indian ocean populations (Madagascar, Mauritius, Tanzania etc.). Such information also could be considered if import livestock of *P. monodon* for culture purposes.

Dissemination/application of research output

Results of the current study have been presented at the Annual Scientific Sessions at National Aquatic Resources Research and Development Agency (NARA) and internationally at the International Conference on Emerging Trends in Scientific Research. To get the sound knowledge through results of this type of studies would be more important to policy makers and relevant personal who are involving in the construction of management and conservation plans of the fisheries and aquaculture industry.

Section 5

Miscellaneous

i) List of major equipment acquired during the project period and their functionality
None

ii) List of publications/communications arising from the project and/or presentations made at seminars, workshops etc. (Please attach copies)

- 'Genetic diversity of tiger prawn (*Penaeus monodon*) populations in Sri Lanka: implications for aquaculture and conservation programs'
  Presented at Nara Scientific Sessions 2013 **(Annex I)**
- 'Availability of morphologically similar, genetically diverge *Penaeus monodon* populations in Sri Lanka'

  Presented at the International Conference on Emerging Trends in Scientific Research **(Annex II).** Received best oral presentation award at the session **(Annex III).**

  The full paper has been submitted for a journal and pending for a decision.

Section 6

Summary Statement of Expenditure (indicate under Personnel, Equipment, Consumables, Travel and Subsistence and Miscellaneous)

Final Financial Statement – Grant: NSF/RG/2010/BT/02

Grantee: Dr. D.H.N. Munasinghe

Category	Funds Received (Rs)	Total Expenditure (Rs)	Funds Available (Rs)
Personnel			
Research Student			
Technical Assistant	252,000 (- 36000)* 216,000		
Other	7052	236,000	-12,948
Equipments Purchased			
Foreign Local	-	-	-
Consumables			
Foreign			
Local	645,000 (+50,000)** 695,000	681,925	13,075
Travel and subsistence	123,300 (-6,500)* 116,800	116,955	-155
Miscellaneous	35,000 (-7,500)* 27,500	26,618	882
Total	1,062,352	1,061,498	854

*Money transferred to consumable vote on request **Money received to consumable vote

Unspent balance of the funds received: Rs. 854.00

Funds Received: Rs: 1,062,352.00

Actual expenditure: Rs: 1,061,498.00

Balance: Rs. 854.00

Cash Imprest/Cash Advance: --

Balance at 24.10.2013: Rs. 854.00

28, 10.13

Bursar/ Accountant

Date

[illegible]

Section 7

i) Grantees' signatures:

Date: 28.07.2014.

ii) Comments of the Head of the Department/signature:

The project has been successfully completed with outcome publications

Head
Department of Zoology
University of Ruhuna
Matara
Sri Lanka

2014/7/31

iii) Head of the Institution's signature:

05/08/2014

Professor Gamini Senanayake
Vice-Chancellor
University of Ruhuna
Matara - Sri Lanka.

Annexes

Annex I

Proceedings of the National Aquatic Resources Research and Development Agency (NARA) Scientific Sessions 2013

Genetic diversity of tiger prawn (*Penaeus monodon*) populations in Sri Lanka: implications for aquaculture and conservation programmes

D.H.N. Munasinghe*

Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka.

The estimation of intra-specific level of diversity of any species is a fundamental for management of natural and cultured populations. Genetic markers have been used for several applications in *P. monodon*, for instance, in the investigation of genetic variability of wild stocks, maintaining genetic characteristics of artificially propagated stocks, genetic improvement of *P. monodon* domesticated stocks through selective breeding programmes. The first population genetic study on *P. monodon* in Sri Lanka was carried out using 588 bp sequence fragment of mitochondrial control gene region. Three populations were selected from Southern (20), Western (22) and Eastern (21) parts of Sri Lanka. High haplotype number was recorded in Western (11) and Eastern (12) populations while low haplotype number (03) was reported from the Southern population. Only one haplotype was common among Southern and Eastern populations and all others were specific to each population. When Sri Lankan haplotypes were compared with other published *P. monodan* sequences for the relevant gene region, results indicated that Sri Lankan haplotypes are closely related with the Western pacific ocean populations (Thailand, Thaiwan, Vietnam etc.) than Western Indian ocean populations (Madagascar, Mauritius, Tanzania etc.). This may be due to the pattern of oceanic currents which contribute to the distribution of the pelagic larval stage of *P. monodon.* However, one haplotype is highly abundant in Southern population which indicates low genetic variation. Occurrence of such types of low genetic variation could be possible due to high exploitation of the available natural population or the population being isolated due to a geographical barrier available in the coastal region. This information could be utilized when selecting brood stocks for *P. monodon* culturing programmes and implementation of management strategies for conservation of wild populations in Sri Lanka.

Keywords: *Penaeus monodon*, Sri Lanka, mitochondrial control gene region, genetic diversity, haplotype

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Annex II

Paper No: ICETSR -107

Tittle: **Availability of Morphologically Similar, Genetically Diverge *Penaeus Monodon* Populations in Sri Lanka**

Author(s): **Munasinghe D.H.N.** (Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka)

Abstract: Morphological and genetic variations of three Penaeus monodon populations in Sri Lanka were determined using data from truss network method and partial amplification of mitochondrial control (mtC) gene region respectively. Total of 37 morphometric characters were collected from 168 individual representing three populations (eastern, western and southern) and subjected to Principle Component Analyses (PCA) to determine the morphological variation among three populations. Two principal components derived from PCA analysis with the accumulated variance for the two PC represented as 75% of the total. The plot against first and the second principal components scores revealed great morphological similarity among three populations.

For genetic analysis, total of 600 bp DNA fragment was amplified from mtC gene region using 63 individuals. Total of twenty five haplotypes were produced for three populations and one haplotype was common between southern and eastern populations. Mean haplotype diversity (h) and nucleotide diversity (π) among three populations were ranged from 0.35 (southern) to 0.92 (eastern) and from 0.5% (southern) to 2.5% (western) respectively. Analysis of molecular variance (AMOVA) indicated within population variation as 78.89% and among population variation as 21.11% indicating high intra-population diversity. The overall Fst value indicated significant genetic structure among three populations (Fst = 0.2111, P = 0.00 < 0.05). When compare results of the present study with the previously published data, it indicates availability of genetically more structured populations in south Indian Ocean than Indo –Pacific region. The possible reasons for the occurrence of morphologically similar but genetically diverged populations in Sri Lankan waters are discussed in the paper.

Keywords: Penaeus monodon, Morphology, truss network, Genetic variation, Mitochondrial control gene region

Annex III

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Best Paper Certificate

This is to Certify that

[illegible]

Has been awarded "Best Research Paper" on presenting paper titled

ICETSR-107: Availability of morphologically similar, genetically diverge Penaeus monodon populations in Sri Lanka

at

International Conference on Emerging Trends in Scientific Research held on March 15-16, 2014 at Pearl International Hotel Kuala Lumpur, Malaysia

organized by

Asian Economic and Social Society
Faculty of Technology Management and Business,
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