Determination of Taxonomic Status and Phylogenetic Affinities of Common Edible Crab Species in Southern Coast of Sri Lanka Using DNA Barcoding Gene Region

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Abstract Edible crabs are among the known and valuable economic sea foods exported, as well as the consumed locally in southern Sri Lanka. However, genetic identification and taxonomic status of available crab species are less studied. This study identified ten selected varieties of edible crab species using information of barcoding gene region. Acquired consensus sequences of partially amplified mitochondrial COI gene region (710bp) were processed in BLAST option of NCBI database. Results identified six different species fallen within three genera, namely *Scylla serrata*, *Scylla olivacea*, *Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis japonica* and *Charybdis natator* which were confirmed with morphological keys. Identified sequences were analyzed with sequences from other geographical regions and Minimum Evolution Tree; sequence divergence levels among sequences were also estimated. Phylogenetic tree produced three separated clades with basal clade of Genus *Portunus*. Two *P. pelagicus* sequences have been linked with Australia and Russia independently, while two *P. sanguinolentus* sequences behaved as haplotypes, diverged slightly from South Korea, India and China. *Scylla serrata* sequences were promising a strong genetic uniformity with India and Thailand. However, in *Scylla olivacea*, two sequences related to India and Thailand separately. Sequence of *Charybdis natator* showed a greater similarity to Japanese sequence while samples collected as *Charybdis japonica* seems to be controversial and need further studies. As the first genetic study conducted to estimate taxonomic status of edible crabs in Sri Lanka, results are important for use in conservation strategies, as well as further research and prospective culturing programs.

Keywords Common Edible Crabs, COI Gene, DNA Barcoding, Southern Sri Lanka, Phylogeny

1. Introduction

It is much reasonable to deem that edible crabs (Crustacea: Decapoda: Portunidae) own an important food value because, they comprise a distinct taste as well as the unique nutritive value. Sri Lanka, being an island, has a coastal line of 1760 km in contact with estuaries and lagoons. These areas are considerably rich in crabs due to the habitat compatibility. Crab fishery may be of two main subsectors, marine crab fishery and lagoon or estuarine crab fishery. Nevertheless, fishers gain their income by both of sectors, especially by fulfilling the demand of overseas.

According to the past records, in Sri Lanka there are three popular species of edible crabs throughout the country. They are in the order of preference *Scylla serrata* (Forsk., 1775), *Portunus pelagicus* (Linnaeus, 1758) and *Portunus sanguinolentus* (Herbst, 1783) [9].

Scylla serrata is commonly known as “lagoon crab or mud crab” as they inhabit mostly the brackish water, usually just offshore on soft muddy bottoms among mangroves and is of great demand, in local and export markets. It is a large crab which can grow up to about 25 cm in carapace length and 1.5 kg in weight [9]. *Portunus pelagicus* is a marine species distributed throughout Indo-West Pacific region of which males are provided with blue markings, and females with dull green, hence referred to as ‘blue swimming crab’. It is found from sandy to sandy-muddy substrates in shallow waters down to a depth of 50 m, including areas near reefs, mangroves, and in sea grass and algal beds [1]. *P. pelagicus* also grows to a fairly large size of about 10 -15 cm in carapace length, and attains a weight of about 500 - 750 g [9]. *Portunus sanguinolentus* is a swimming crab, coloured olive to dark green, with 3 prominent maroon to red spots on posterior 1/3 of carapace which can grow up to maximum carapace width of 20 cm. They inhabit sandy to sandy-muddy substrates, from the intertidal zone (especially juveniles) to depths of 30 m. Although distribution is the

same, this species is less common than *Portunus pelagicus*, and appears only occasionally in markets. It is priced similarly to, or for slightly lower prices as, *P. pelagicus* [1].

Genomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms [14]. Studies evaluated the potential of using COI as a taxonomic tool for species identification [7]. DNA barcoding has become a promising tool for rapid and accurate identification of various taxa and to reveal unrecognized species in several animal groups. Moreover, the introduction of DNA barcoding has highlighted the expanding use of the COI as a genetic marker for species identification [3].

Scylla serrate, commonly known as the giant mud crab, has been studied using DNA sequences by Fuseya and Watanabe [4]; Keenan *et al.* [11] and Gopurenko and Hughes [6]. Costa *et al.* [2] has highlighted the use of barcoding region of mitochondrial genome for the analysis of genetic data for crustaceans.

Past studies have pointed out that the identification of *Scylla* species using morphological data is controversial; especially, in larval and juvenile (crablet) stages [5]. Besides, *P. pelagicus* may be confused with *P. trituberculatus*, which resembles a large stocky female of *P. pelagicus* [1]. Therefore, utility of genetic information was recommended for identification of crab species [4]. This approach is more advantageous in crab fattening industry in which the larval stages are collected from the wild and reared them up to adult stage.

In addition to 3 popular species, i.e. *Scylla serrata*, *Portunus pelagicus* and *P. sanguinolentus*, there are many edible crab varieties available in Southern Sri Lanka, for which the exact taxonomic statuses are not determined. On the otherhand past studies based on morphological cues and molecular level identification of commercially important crab species has not been reported in Sri Lanka. Thus, there were two objectives of this study: to collect barcoding data of common edible crab species recorded in the southern coastal region of Sri Lanka and to determine their species status and phylogenetic affinities paired with relevant species recorded from other geographical regions of the world using data of the DNA barcoding gene region.

2. Methods and Materials

2.1. Collection of Specimens and Preservation

Edible crab samples were collected from several different fish landing sites and fish markets along the southern coastal belt of Sri Lanka, including Bentota, Balapitiya, Ambalangoda, Dodanduwa, Mahamodara, Galle, Habaraduwa, Weligama, Polwathumodara, Thotamuna (Matara), Pehembiya (Dikwella), Rekawa, Kahandamodara and Yala (Figure 1).

Figure 1. Sample collection sites of the current study, along the southern coastal belt of Sri Lanka

During the collection process, at least three individuals from each variety at each site were collected including both sexes to the greatest extent possible. All collected species were identified using morphological characteristics according to the published keys for crustaceans [1].

After the morphometric categorization, minimum of two to three individuals from each morph; regardless of the site, were selected for the analysis. Seventeen selected specimens were dissected in order to acquire a muscle sample of 3-5 g with minimum damage to their body. Flesh samples were preserved in absolute ethanol. Remaining specimens were kept under cryogenic conditions at the Research Laboratory, Department of Zoology, University of Ruhuna, Matara, Sri Lanka as reference materials.

2.2. DNA Extraction, PCR Amplification and Sequencing

DNA extraction was carried out using QIAGEN DNeasy Blood & Tissue Kit (USA). Partial amplification of around 710-bp fragment of mitochondrial COI (Cytochrome Oxidase Subunit I) gene region was performed using primers, LCO1490 and HCO2198. LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGT GACCAAAAATCA-3' (Folmer *et al.*, 1994). Polymerase Chain Reaction was carried out in 25 µL reaction volumes comprised of 5.0 µL of template DNA extract, 2.5 µL of 10X PCR buffer, 2.5 µL of 15 mM MgCl₂, 2.5 µL of 2 mM of each dNTP, 2.0 µL of 10 µM each forward and reverse primers, 2.0 µL of Taq polymerase and 6.5 µL of PCR water.

Thermal cycle for the reaction consisted of 2 minutes initial denaturing phase at 94 °C, further 30 seconds at 94 °C, 30 seconds primer annealing phase at 46 °C, 1 minute elongation phase at 72 °C and final extension phase at 72 °C for 7 minutes. PCR amplicons which produced clear bands in agarose gel electrophoresis were sent to Macrogen Pvt. Ltd, South Korea for purification and sequencing.

2.3. Analyses of Data

2.3.1. Determination of Species Status of Selected Specimens

Acquired sequences were aligned, edited and finally consensus sequences were obtained with the aid of the BioEdit-Sequence Alignment Editor software package. Consensus sequences of each morph were compared with the maximum compatibility and similarity of pre-published sequences using the BLAST option (Basic Local Alignment Search Tool) of National Center for Biotechnology Information (NCBI) official database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Identified species were morphologically compared and confirmed using pre published reference materials [1].

2.3.2. Publication of DNA Barcoding Data

After clarification of the species status of samples collected, barcoding sequences were published in the data-base of National Center for Biotechnology Information (NCBI) and Barcode of Life Database (BOLD).

2.3.3. Determination of Phylogenetic Status of Identified Species

After justification of the species status, related sequences published in the Genbank from other geographical regions for each species were downloaded for analyses (Table 1).

Phylogenetic analyses were performed using MEGA 5.1 program. The Minimum Evolution tree was generated to estimate the strength of the phylogenetic relationship by taking 1000 as the bootstrap value. *Hepatoporus orientalis* (Sakai, 1935) (Crustacea, Decapoda, Brachyura, Xanthidae) was used as the out group taxa (GenBank: HM750994.1). Pairwise distance table ('P' distance) was constructed using the same options used for the estimation of the tree.

Table 1. Details of sequences downloaded from NCBI, published for relevant species in other geographical regions.

NCBI accession number.	Species	Country, recorded	Abbreviations in the tree
FJ827758.1	<i>Scylla serrata</i>	Thailand	S.ser (5) THL
KF612462.1	<i>Scylla serrata</i>	India	S.ser (6) IND
JN085429.1	<i>Scylla serrata</i>	India	S.ser (7) IND
KC200562.1	<i>Scylla serrata</i>	India	S.ser (8) IND
FJ827760.1	<i>Scylla olivacea</i>	Thailand	S.oli (11) THL
KC200563.1	<i>Scylla olivacea</i>	India	S.oli (12) IND
FJ812293.1	<i>Portunus pelagicus</i>	Australia	P.pel (23) AUS
JX398106.1	<i>Portunus pelagicus</i>	Russia	P.pel (24) RUS
EU284144.1	<i>Portunus sanguinolentus</i>	China	P.san (29) CHN
KC760157.1	<i>Portunus sanguinolentus</i>	India	P.san (30) IND
JX502943.1	<i>Portunus sanguinolentus</i>	South Korea	P.san (31) SK
EU586120.1	<i>Charybdis japonica</i>	China	C.jap (19) CHN
HM237597.1	<i>Charybdis japonica</i>	China	C.jap (18) CHN
HM237598.1	<i>Charybdis japonica</i>	China	C.jap (17) CHN
KF793328.1	<i>Charybdis natator</i>	Japan	C.nat (22) JAP

3. Results

3.1. Determination of Species Status of Several Crab Morphs

BLAST results revealed that selected specimens belong to seven different species within three genera namely, *Scylla serrata*, *Scylla olivacea* (Herbst, 1796), *Scylla tranquebarica* (Fabricius, 1798), *Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis japonica* (A. Milne Edwards, 1861) and *Charybdis natator* (Herbst, 1789). However six species were confirmable without *S. tranquebarica*.

3.2. Publication of DNA Barcoding Data

Reference numbers for all sequences which were clearly identified and submitted to NCBI and BOLD Systems are given in Table 2.

Table 2. Reference numbers of the NCBI and BOLD system for sequences derived from the current study.

Sample identity/ Abbreviation	NCBI accession number	BOLD Systems accession number
<i>Charybdis natator</i> 1 / 20.SSL	KM528124	SLCRB001-14
<i>Charybdis natator</i> 2 / 21.SSL	KM528125	SLCRB002-14
<i>Portunus pelagicus</i> 1/ 25.SSL	KM528127	SLCRB005-14
<i>Portunus pelagicus</i> 2 / 26.SSL	KM528126	SLCRB006-14
<i>Portunus sanguinolentus</i> 1 / 27.SSL	KM528128	SLCRB007-14
<i>Portunus sanguinolentus</i> 2 / 28.SSL	KM528129	SLCRB008-14
<i>Scylla olivacea</i> 1 / 9.SSL	KM528131	SLCRB003-14
<i>Scylla olivacea</i> 2 / 10.SSL	KM528130	SLCRB004-14
<i>Scylla serrata</i> 1 / 1.SSL	KM528136	SLCRB009-14
<i>Scylla serrata</i> 2 / 2.SSL	KM528132	SLCRB010-14
<i>Scylla serrata</i> 3 / 3.SSL	KM528135	SLCRB011-14
<i>Scylla serrata</i> 4 / 4.SSL	KM528134	SLCRB012-14

3.3. Determination of Phylogenetic Status of Identified Species

Minimum evolution tree acquired in MEGA program is shown in Figure 2. Estimated sequence divergence levels among taxa obtained in this study are given in Table 3.

SSL - Southern Sri Lanka, JAP - Japan, SIN - Singapore, THL - Thailand, AUS - Australia, RUS - Russia IND - India, CHN - China, SK - South Korea

Figure 2. Minimum Evolution Tree derived for partial COI mitochondrial gene region of selected crabs from southern Sri Lanka and other geographical regions.

Table 3. Sequence divergence levels ('P' distances) among in-group and out-group species for partially amplified COI mitochondrial gene region

	1.SSL	2.SSL	3.SSL	4.SSL	5.THL	6.IND	7.IND	8.IND	9.SSL	10.SSL	11.THL	12.IND	13.SSL	14.SSL	15.SSL	16.SSL
1.SSL																
2.SSL	0.000															
3.SSL	0.000	0.000														
4.SSL	0.000	0.000	0.000													
5.THL	0.000	0.000	0.000	0.000												
6.IND	0.000	0.000	0.000	0.000	0.000											
7.IND	0.000	0.000	0.000	0.000	0.000	0.000										
8.IND	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
9.SSL	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114								
10.SSL	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.006							
11.THL	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.006	0.000						
12.IND	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.114	0.000	0.006	0.006					
13.SSL	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.167	0.161	0.161	0.167				
14.SSL	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.167	0.161	0.161	0.167	0.006			
15.SSL	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.202	0.202	0.202	0.202	0.158	0.152		
16.SSL	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.202	0.202	0.202	0.202	0.158	0.152	0.000	
17.CHN	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.158	0.161	0.161	0.158	0.155	0.150	0.103	0.103
18.CHN	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.158	0.161	0.161	0.158	0.155	0.150	0.103	0.103
19.CHN	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.158	0.161	0.161	0.158	0.155	0.150	0.103	0.103
20.SSL	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.176	0.179	0.179	0.176	0.182	0.176	0.158	0.158
21.SSL	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.176	0.179	0.179	0.176	0.182	0.176	0.164	0.164
22.JAP	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.176	0.176	0.173	0.176	0.170	0.158	0.158
23.AUS	0.196	0.196	0.196	0.196	0.196	0.196	0.196	0.196	0.196	0.208	0.205	0.205	0.208	0.199	0.194	0.179
24.RUS	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.202	0.199	0.199	0.202	0.194	0.188	0.176
25.SSL	0.199	0.199	0.199	0.199	0.199	0.199	0.199	0.199	0.205	0.202	0.202	0.205	0.182	0.176	0.179	0.179
26.SSL	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.205	0.205	0.208	0.196	0.191	0.182	0.182
27.SSL	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.220	0.217	0.217	0.220	0.208	0.208	0.191	0.191
28.SSL	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.220	0.220	0.220	0.220	0.211	0.211	0.185	0.185
29.CHN	0.199	0.199	0.199	0.199	0.199	0.199	0.199	0.199	0.214	0.214	0.214	0.214	0.199	0.199	0.179	0.179
30.IND	0.205	0.205	0.205	0.205	0.205	0.205	0.205	0.205	0.214	0.214	0.214	0.214	0.199	0.199	0.179	0.179
31.SK	0.202	0.202	0.202	0.202	0.202	0.202	0.202	0.202	0.217	0.217	0.217	0.217	0.205	0.205	0.185	0.185
32.SIN	0.472	0.472	0.472	0.472	0.472	0.472	0.472	0.472	0.493	0.493	0.493	0.493	0.499	0.499	0.472	0.472

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in Southern Coast of Sri Lanka Using DNA Barcoding Gene Region

	17.CHN	18.CHN	19.CHN	20.SSL	21.SSL	22.JAP	23.AUS	24.RUS	25.SSL	26.SSL	27.SSL	28.SSL	29.CHN	30.IND	31.SK	32.SIN
1.SSL																
2.SSL																
3.SSL																
4.SSL																
5.THL																
6.IND																
7.IND																
8.IND																
9.SSL																
10.SSL																
11.THL																
12.IND																
13.SSL																
14.SSL																
15.SSL																
16.SSL																
17.CHN																
18.CHN	0.000															
19.CHN	0.000	0.000														
20.SSL	0.147	0.147	0.147													
21.SSL	0.147	0.147	0.147	0.006												
22.JAP	0.141	0.141	0.141	0.006	0.006											
23.AUS	0.182	0.182	0.182	0.191	0.188	0.185										
24.RUS	0.182	0.182	0.182	0.211	0.208	0.205	0.053									
25.SSL	0.182	0.182	0.182	0.202	0.199	0.196	0.047	0.032								
26.SSL	0.185	0.185	0.185	0.191	0.188	0.185	0.018	0.059	0.044							
27.SSL	0.199	0.199	0.199	0.202	0.205	0.202	0.185	0.176	0.179	0.182						
28.SSL	0.191	0.191	0.191	0.191	0.196	0.191	0.191	0.182	0.185	0.188	0.012					
29.CHN	0.191	0.191	0.191	0.182	0.188	0.182	0.188	0.179	0.182	0.185	0.021	0.015				
30.IND	0.188	0.188	0.188	0.185	0.191	0.185	0.191	0.182	0.185	0.188	0.021	0.015	0.006			
31.SK	0.194	0.194	0.194	0.191	0.196	0.191	0.194	0.185	0.188	0.191	0.023	0.018	0.009	0.009		
32.SIN	0.475	0.475	0.475	0.501	0.499	0.496	0.475	0.463	0.463	0.472	0.499	0.496	0.490	0.490	0.490	

Refer Table 1 and 2 for abbreviations.

4. Discussion

4.1. Determination of Species Status of Several Crab Morphs

Among derived sequences, except two, others have been identified according to the blast results and revealing selected specimens belong to seven different species categorized under three genera. The species status of sequences results for *Scylla tranquebarica* have not been confirmed hence, have not been utilized for phylogenetic analysis. This was because only one sequence existed, which is not sufficient to generate conclusions. On the other hand, its identification in BLAST was also vague.

4.2. Determination of Phylogenetic Status of Identified Species

According to the Minimum Evolution Tree (Figure 2) obtained, three different clades have clearly deviated according to genera, Genus *Scylla*, Genus *Portunus*, and Genus *Charybdis*. Separation of different clades revealed that Genera *Scylla* and *Charybdis* together exhibit more distant relation from Genus *Portunus*. Estimated sequence divergence levels among taxa used in this study are given in the Table 3.

With the aid of the level of divergence, it can be shown that *Scylla serrata* recorded from India, Thailand and southern Sri Lanka are of a greater genetic similarity which is further supported by a 100% bootstrap value. Yet, *S. olivacea* behaves in a different manner in which two sequences illustrate similarities with two different downloaded sequences. i.e. One specimen 10.SSL (refer to Table 2 for sequence identities) is compatible with the sequence reported from Thailand with a zero divergence. While additionally, specimen 9.SSL, is in connection with the Indian sequence with the same zero divergence. The bootstrap node support of these two varieties of *S. olivacea* is 100% (refer to Figure 1) and connections of each pair of compatible sequences are 88% of node support. This separation is a bit doubtful and further analysis is necessary. However, this might be resulted due to introduction of different varieties into Sri Lankan waters from anthropogenic or natural causes. Percentage divergence between these two *Scylla* species (11.4%) is greater than 0.02 (2%) - the interspecific genetic distance, according to Hebert *et al.* [8]. Therefore, their species identity is well pronounced. It can also be stated that southern Sri Lankan *S. olivacea* is related both to Indian and Thai species.

The clade named genus *Charybdis*, is comprised of two species, *C. japonica* and *C. natator* with a magnitude of divergence above 15%. Two *C. natator* sequences behaved in a compatible manner with a very low divergence level of 0.006. Those two sequences are in close relation in same 0.006 divergences with the Japanese variety. This reveals that there might be a good flow of genes in between Japan and Sri Lanka in the case of *C. natator*.

However, *C. japonica* exhibited a complex status. There are two varieties identifiable in colour pattern, but resulted in the same species according to the blast results. The phylogenetic tree shows that those two morphs have been separated clearly, i.e. 13.SSL and 14.SSL from 15.SSL and 16.SSL. Moreover, those two varieties are separated from reported Chinese sequences, 15 and 16 with 10% divergence and 13 and 14 with 15%. Apart from that the two identified Sri Lankan morphs produce a divergence of 15.8%. Therefore, their species level determination as the same species becomes controversial. Perhaps those varieties may belong to different species of the same genus or sub-species of same species as the morphology of two morphs also seems to be dissimilar. According to the results it can also be suggested the availability of cryptic species in the genus.

The last distinct clade, which consisted of Genus *Portunus*, has been conclusively subdivided into the two species *P. pelagicus* and *P. sanguinolentus*. On the other hand, Genus *Portunus* can be considered as the basal clad which produces two sister-clades of genera *Charybdis* and *Scylla*. Two specimens of *P. pelagicus* are in relation with the sequences of two different geographical regions, Australia and Russia from which the divergence level of those two Sri Lankan sequences was recorded as 4%.

Two sequences of *P. sanguinolentus* from southern Sri Lanka show a divergence level of 1.2%. Therefore, it can be confirmed that those are two haplotypes of the above species. Apart from that *P. sanguinolentus* sequences show a relation with South Korea, India and China with fairly low divergence level of approximately 2%. Thus, it can be confirmed that *P. pelagicus* and *P. sanguinolentus* are distributed in Indo-Pacific region with more or less similarities with sequences from those countries.

The classification for *Scylla* has been controversial since long. Ultimately, Keenan *et al.* [11] extensively revised the taxonomy of genus *Scylla* using both morphometric and genetic approaches, and divided this genus into four distinct species: *S. serrata*, *S. olivacea*, *S. tranquebarica* and *Scylla paramamosain*. Srinivasagam *et al.* [13] and Kathirvel *et al.* [10] reported the presence of only two species i.e. *S. serrata* and *S. tranquebarica* in Indian waters. Presence of *S. serrata* along the south coast has been reported [12], but this study was able to identify the presence of three species of genus *Scylla* including *S. olivacea* in Southern Sri Lanka.

As observed, morphological variations are distinguishable among three of those species. *S. serrata* is more or less greyish-green in body colour where *S. olivacea* contains reddish-brown together with the dark green. The specimen identified as *S. tranquebarica* is more or less dark brown. Abdomen is clearly striped with brown coloured bands. It is important to consider the presence of the *S. tranquebarica* in association with *S. olivacea* as recorded by Keenan *et al.* [11]. Confirmation of the specimen, which resulted as *S. tranquebarica* is bit difficult, because only one specimen was encountered. Therefore, further collection of samples and analysis of data are essential in order to draw better conclusions.

It is important to investigate the diversity of faunal groups and produce information in a standard way to internationally accepted data-bases, which can be utilized by other research. The current study published 12 sequences in databases NCBI and BOLD, among them KM0528130/SLCRB004-14, KM528131/SLCRB003-14, KM528124/SLCRB001-14 and KM528125/SLCRB002-14 are unique and have not been recorded previously in Sri Lanka. According to the current study, the most abundant mud crab species in southern Sri Lanka is the *S. serrata*, when compared to other two species. Hence, it fetches the highest economic consideration due to the better taste as well. Further, as it was not documented, it is evidence of the availability of *S. olivacea* and *S. tranquebarica* in southern coastal region of Sri Lanka. Mud crab fisheries have been functioning in a considerable status in the country for several decades. With the high demand in and export of crab, this sector generates international economic activity, as well as and constitutes an important means of income generation for local riparian communities. However, the increase in coastal populations, the high demand for mud crabs from foreign countries, the ease of collection of in shallow coastal waters by traps, gill nets, scoop nets, hand picking and the introduction of fattening for soft shell ones, have all combined to cause overfishing of this valuable resource. In spite of the importance of mud crabs, information of their biology and ecology which is crucial for management is still in an undervalued condition in Sri Lanka.

Gained information, together with species status and their phylogenetic cognitions, may have a greater application in the development of the fisheries sector. There is also a greater utility in conservation and management practices of crab populations.

5. Conclusions

In the final analysis, it is evident that six different species of commonly available edible crabs are found along the southern coastal belt of Sri Lanka. However, three genera were suggested to inhabit in the study area with the species identity of *Scylla serrata*, *Scylla olivacea*, *Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis japonica* and *Charybdis natator*.

Concerning the divergence level, it can be concluded that *Scylla serrata* recorded from India and Thailand are in a greater genetic uniformity with the southern Sri Lankan specimens. However, *Scylla olivacea* behaved differently, one specimen was in genetic cognition with Thailand, and the other one with India.

In the case of *Charybdis natator*, it can be concluded that recorded specimen are in a strong relation with Japanese sequence. However, the phylogenetic status of suggested *C. japonica* samples is controversial and need further analysis for a better conclusion.

Genus *Portunus* is the basal clade which has produced other sister clads. By conferring with the relationships, the Indo-West Pacific distribution of Genus *Portunus* can be accepted.

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REFERENCES

- [1] K. E. Carpenter, V. H. Niem. FAO Species identification guide for fishery purposes; the living marine resources of the western central pacific. Food and Agriculture Organization of the United Nations, Rome. 1998. Volume 2. 1115-1130
- [2] F. O. Costa, J. R. de Waard, S. Ratnasingham, R. T. Dooh, M. Hajibabaei, P. D. N. Hebert. Biological identification through DNA barcodes: the case of the crustacea. Canada Journal of Fisheries and Aquatic Sciences. 64: 272-295, 2007.
- [3] N. Dawnay, R. Ogden, R. McEwing, G. R. Carvalho. Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Science International. 173: 1-6, 2007.
- [4] R. Fuseya, S. Watanabe, Genetic variability in the mud crab genus *Scylla* (Brachyura: Portunidae). Fisheries Science. 62: 705-709, 1996.
- [5] H. Fushimi, S. Watanabe. Problems in species identification of the mud crab Genus *Scylla* (Brachyura: Portunidae). US-Japan Natural Resources Technical Report. 28: 09-13, 2008.
- [6] D. Gopurenko, J. M. Hughes. Regional patterns of genetic structure among Australian populations of the mud crab, *Scylla serrata* (Crustacea: Decapoda): evidence from mitochondrial DNA. Marine and Freshwater Research. 53: 849-857, 2002.
- [7] P. D. N. Hebert, A. Cywinska, S. L. Ball, J. R. deWaard. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. 270: 313-321, 2003.
- [8] P. D. N. Hebert, S. Ratnasingham, J. R. deWaard. Barcoding animal life: Cytochrome oxidase subunit I divergences among closely related species. Proceedings of the Royal Society of London. 270: 96-99, 2003.
- [9] S. C. Jayamanne. Crab resource of Sri Lanka. 'Vidu', the magazine published by National Aquatic Resource Agency, Sri Lanka, 2011.
- [10] M. Kathirvel, S. Kulasekhar, C. P. Balasubramaniam. Mud crab culture in India. Central Institute of Brackish water Aquaculture (ICAR). 17: 60, 2004.

- [11] C. P. Keenan, P. J. F. Davie, D. L. Mann. A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). The Raffles Bulletin of Zoology. 46(1): 217–245, 1998.
- [12] P. Maitipe, S. S. De Silva. The structure and function of krall, a fishing gear, in a Sri Lanka lagoon. Journal of Fish Biology. 33(1): 137-143, 1896.
- [13] S. Srinivasagm, M. Kathirvel, S. K. Pandian. Captive, brood stock development induced breeding and larval stages of mud crabs (*Scylla* sp.). Central Institute of Brackish water Aquaculture (ICAR), India. 12: 26, 2000.
- [14] K. M. Wilson. Molecular biology as a tool for taxonomy. *Clinical Infectious Diseases*. 20:192-208, 1995.