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Mitochondrial DNA variation and population genetic structure of white shrimp *Fenneropenaeus indicus* along the coastal belt of Sri Lanka⋆

M.D.S.T de Croos[a] and Snæbjörn Pálsson

Institute of Biology, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland

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Abstract – The white shrimp (*Fenneropenaeus indicus*) is an ecologically and economically important penaeid species, widely distributed in the Indo-Pacific region. To obtain information on the genetic variation and population structure of *F. indicus,* sequencing analysis was conducted on a fragment of cytochrome oxidase subunit I (COI) of the mitochondrial DNA. Individuals (n = 217) from eight locations covering four main geographic regions along the coastal belt of Sri Lanka were analysed. The sequences, a 602-base pairs (bp) fragment in length, revealed high haplotype and nucleotide diversity that yielded 82 haplotypes. A mismatch analysis produced a unimodal distribution of pairwise differences between haplotypes, consistent with a historic rapid population expansion. Little or no genetic differentiation was observed between most samples, although genetic distances based on pairwise differences between haplotypes started to increase when geographic distances exceeded about 130 km. A population in Bundala (southeast) had lower diversity and was genetically differentiated from the others. This information could be important for the sustainable management and utilization of this resource.

Key words: Penaeidae / *Fenneropenaeus indicus* / COI mitochondrial DNA / Genetic diversity / Population structure / Indian Ocean

Introduction

The assessment of population genetic diversity and structure is important for maintaining productive fisheries and sustainable harvesting of populations (Park and Moran 1994; Begg et al. 1999; Thorrold et al. 2002). Genetic diversity can reflect changes in population size; a population decline can thus be revealed by decreased genetic diversity (e.g. Glenn et al. 1999). Assessments of intraspecific genetic diversity and population genetic structure provide useful additional information for taxonomic and evolutionary history studies (Benzie et al. 1995) and for conservation and aquaculture of a species (Moore et al. 1999; Klinbunga et al. 2001; Valles-Jimenez 2006).

Shrimps of the family Penaeidae are an ecologically diverse group of species that inhabit estuarine and marine environments throughout many of the world's oceans (Gulland and Rothschild 1984). Penaeids can represent a valuable fisheries resource and are extensively cultured (Leung and Engle 2006). The ecological and economic importance of these shrimps has led to much biological and genetic research. Penaeids show a wide range of patterns of intraspecific population genetic structure: some researchers have reported the absence of distinguishable population structures over thousands of kilometers (Benzie 2000; Brooker et al. 2000; McMillen-Jackson and Bert 2004a; Cui et al. 2007), while others report significant structural differentiation over hundreds of kilometres (Klinbunga et al. 1999; You et al. 2008). According to Benzie (2000), most of the genetic structure observed in penaeids is due to historic events, and can be maintained by present-day barriers to gene flow (Aubert and Lightner 2000; Garcia-Machado et al. 2001). Among the species in the Penaeidae family, eight are considered to be economically important; *Penaeus monodon, P. merguiensis, Fenneropenaeus indicus, P. stylirotris, P. chinensis, P. vannamei, P. japonicus,* and *Metapenaeus ensis* (Leung and Engle 2006).

Throughout the coastal regions stretching eastwards from the south and east African coasts to the western Pacific Ocean, including the Red Sea, Madagascar, India, Sri Lanka, China, the Philippines and northern Australia, *Fenneropenaeus indicus* (previously known as *Penaeus indicus*, H. Milne Edwards 1837) is present in inshore and offshore habitats (Fischer and Bianchi 1984; Mohan and Siddeek 1995). *F. indicus* is an ecologically and economically important species in Sri Lankan fisheries (Fischer and Bianchi 1984; Davenport et al. 1999;

⋆ Supporting information is only available in electronic form at www.alr-journal.org

[a] Corresponding author: dileepa_dc@yahoo.com

Jayawardena et al. 2004) and in many other regions of the world.

Different distributions of *F. indicus* life history stages have been reported in lagoons, estuaries and adjacent coastal waters close to the shore, at up to 15–30 m water depth in Sri Lankan waters, based on fishery dependent surveys (Jayawardena 2002a,b; De Bruin et al. 1994). Adults spawn offshore, pelagic larvae migrate inshore, and juveniles spend several months in mangroves and estuaries before migrating offshore (Dall et al. 1990). As in most benthic invertebrates, *F. indicus* larvae require appropriate physical processes to migrate to their adult habitats, such as surface transport generated by direct winds, current forcing (Hawkins and Hartnoll 1982), internal waves (Shanks 1983) and internal tidal bores (Pineda 1994). The patterns of these physical processes are not always explainable and are often complicated by large interannual and spatial differences (Shanks 1983; Pineda 1999). As a result, differences in recruitment, larval retention (Shanks and Eckert 2005) and population structure (Palumbi 2003) may occur.

The complex life history and physical oceanographic factors limit the use of traditional approaches, such as using physical tracking of individuals, for the characterization of populations. Many molecular genetics studies have been carried out to characterize the population genetic structures of marine organisms, but few studies have focused on shrimps, crabs or lobsters despite their abundance (~9000 species in order Decapoda) and distribution (McMillen-Jackson and Bert 2004b).

Panmictic populations are common in coastal invertebrates with planktonic larvae, and such a development strategy promotes continuing gene flow between locations that in close proximity (Avise 2000). Genetic analysis of marine population structures therefore often indicates only slight geographic differentiation in species with high dispersal potential (Palumbi 2003), although recent studies have reported genetic differences over just a short distance (Grosberg and Cunningham 2001; Couceiro et al. 2007; Hellberg 2009). Mitochondrial DNA has been extensively used as a genetic marker in shrimp species (Garcia-Machado et al. 2001; Klinbunga et al. 2001; McMillen-Jackson and Bert 2004a) and has proved to be useful in several population genetic and phylogeographic studies due to several favorable features, such as non-recombination, maternal inheritance, and high mutation rate (Castro et al. 1998).

The aim of this study is twofold: firstly, to provide genetic information, as diversity and stock structure information will allow fishery managers in Sri Lanka to make sound management decisions and regulations for harvesting *F. indicus*; and secondly, to obtain genetic information on the species that will allow genetic comparisons between specimens sampled throughout the species range. The current study focuses on the population genetic structures of the COI mtDNA gene in *F. indicus,* to see whether it confirms the presence of a large continuous population or discrete, genetically distinct small subpopulations along the coastal belt of Sri Lanka.

This study presents the first systematic genetic analysis conducted on a marine invertebrate in the waters of Sri Lanka.

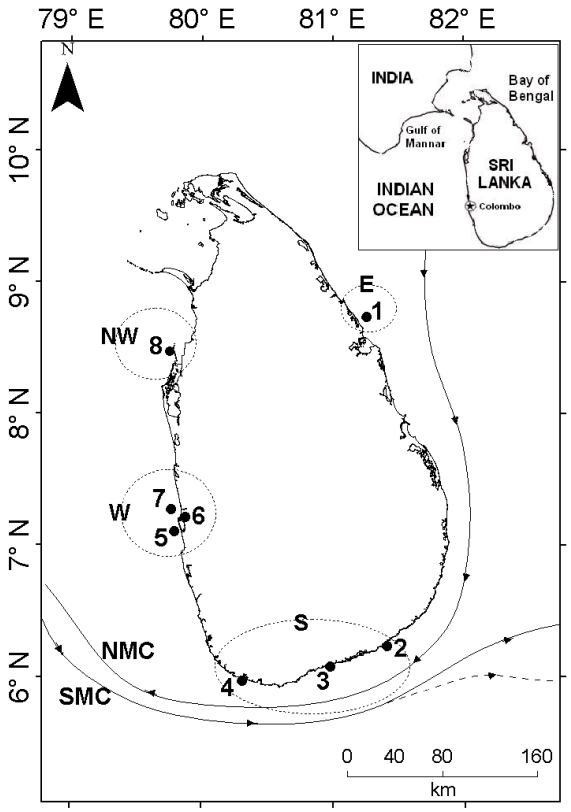

Fig. 1. Location of sampling sites along the coast of Sri Lanka. Names of these sampling sites are given in Table 1, with their respective latitudes and longitudes. Regions are denoted NW = northwest, W = west, S = south and E = east. Currents indicated are the southwest and northeast Monsoon Currents (SMC and NMC).

Table 1. Sample locations of *Fenneropenaeus indicus.*

Site	Location	Latitude, longitude (decimal format)
1	Trincomalee	8.5519, 81.2497
2	Bundala	6.2138, 81.2250
3	Rakawa	6.0500, 80.8500
4	Koggala	5.9859, 80.3384
5	Hendala	6.9929, 79.8040
6	Negombo (lagoon)	7.1540, 79.8085
7	Negombo (coast)	7.2086, 79.8317
8	Kalpitiya	8.2529, 79.7274

Materials and methods

Sampling and DNA extraction

A total of 217 individual adult and juvenile *F. indicus* shrimps (101 males and 116 females), ranging in size from 23 mm to 68 mm carapace length, were collected from 8 locations along the coastline of Sri Lanka in 2008 and 2009 (Table 1, Fig. 1); the small sample from Trincomalee was obtained only in 2009 due to prevailing conflict on the east coast.

Established commercial fisheries exist in all the sampling sites, due to the abundance of the shrimp populations. Population samples were collected from three locations off the western coast (Negombo, Negombo lagoon and Hendala), three off

the southern coast (Koggala, Rakawa and Bundala), one from the northwestern coast (Kalpitiya) and one from the east coast (Trincomalee). Through observations and interviews with fishers, it was confirmed that all the shrimps were caught by commercial trawling, gillnetting and brush piles in neighboring coastal and lagoon waters of the area and were neither imported nor produced by aquaculture. Pleopod muscle tissue samples were collected from shrimp on ice and immediately stored in 90% ethanol.

Total DNA was extracted from ~1 mg pleopod tissue from the ethanol-preserved samples. Tissue was incubated in 6% Chelex with proteinase K (0.2 mg ml^{-1}) at 64 °C overnight with 650 rpm shaking. PCR amplifications of the COI mtDNA region were made using LCO-1490 and HCO-2198 primers (Folmer et al. 1994). PCR reactions were performed in a final volume of 10 µl, containing 0.75 µl dNTP (0.2 mM), 1.15 µl 1% Tween 20, 1 µl Taq polymerase (5 $U\mu l^{-1}$), 1 µl 0.5 BSA (0.5 mM) and 1 µl DNA (75–150 ng μl^{-1}). The DNA concentration was measured using a ND-1000 spectrophotometer (NanoDrop V 3.5). A thermal cycle was used with following profile: 4 min at 94 °C for 30 s, then 40 cycles of 1 min at 94 °C denaturation, 30 s at 45 °C for annealing and 1 min at 72 °C for extension. A final step of 6 min at 72 °C was added to be sure of complete extension of the fragments. The products were sequenced on ABI 3100 Genetic Analyser (Applied Biosystems) for both forward and reverse directions. Sequences were aligned by eye, using the BioEdit Sequence Alignment Editor program (Hall 1999), and trimmed to 602 base pairs.

Genetic diversity

Genetic variation is summarised in Table 1, including gene diversity (h) – the probability that two randomly chosen haplotypes are different – and nucleotide diversity (π) – the probability that two randomly chosen homologues nucleotides are different within samples. The statistics were calculated using ARLEQUIN, version 3.1 (Excoffier et al. 2006).

The evolutionary relationships between haplotypes were examined with Network software (http://fluxus-engineering.com/), using a median-joining algorithm to build an unrooted cladogram. The outcome was reconstructed using the software package R (http://www.r-project.org) to show, in addition, the main geographical origins where the haplotypes were sampled.

Analysis of population genetic structure

Due to small sample size, the sample from Trincomalee was omitted from the analysis of population structure. Population subdivision of the genetic variation based on pairwise distances among haplotypes was inferred using spatial analysis of molecular variance (SAMOVA 1.0) (Dupanloup et al. 2002). The method is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance due to differences between a given number of groups of populations. The significance level was evaluated by 1000 permutations of populations among groups (Dupanloup et al. 2002). Pairwise distances F_{st} and Φ_{st} were also calculated, by applying conventional F-statistics based on haplotype frequencies and the corresponding Φ statistics, taking pairwise distances among haplotypes into account (Tamura and Nei 1993). Significance was tested by 1000 permutations of the dataset.

The increased probability of rejecting the null hypotheses of no differentiation due to multiple comparisons (k) was considered using the Bonferroni adjustment, but because the Bonferroni adjustment assumes orthogonal comparisons, and is therefore highly conservative (e.g. Sokal and Rohlf 1995), the uncorrected p-values are presented here.

To determine whether the extent of genetic differentiation was correlated with the geographic distance separating populations along the coastline, the Mantel test (Mantel 1967) was used, with 1000 permutations, using the statistical software package R.

Analysis of demographic history

Demographic histories were investigated using the ARLEQUIN package. This method is based on the distribution of the observed pairwise nucleotide site differences (also called the mismatch distribution), and the expected values (for no recombination) in growing and declining populations, as suggested by Rogers and Harpending (1992). A rapidly-growing population is characterised by a unimodal distribution, as accumulation of new mutations is greater than loss due to random genetic drift. In stable populations or admixed populations, the distribution may appear multimodal. The model is based on three parameters: θ initial (before the population growth or decline), θ final (after population growth or decline) and τ, the date of growth or decline measured in units of mutational time ($\tau = 2\ \mu\ t$; t is the time in generations, and μ is the mutation rate per sequence and per generation (Rogers and Harpending 1992). Generation time was assumed to be one year. A molecular clock of 1.4–2.2% sequence divergence per million years was used from the estimate made for the same portion of the mitochondrial COI gene in the shrimp genus *Alpheus* and a reliably-dated geological barrier: the Isthmus of Panama (Knowlton and Weigt 1998).

To evaluate the deviation from neutrality of the observed variation, Tajima's test, Fu's Fs, the Ewens-Watterson test and Chakraborty's test, implemented in ARLEQUIN, were applied and tested using 1000 permutations.

Results

Genetic diversity

Sequences of a 602 bp region were obtained from 217 shrimps, defining 82 haplotypes. Seven types were common and found in 71, 23, 12, 11, 8 and 5 individuals. These dominant haplotypes were found in almost all samples (Fig. 2 and Supplementary Table 1). Nine haplotypes were found in two individuals, and the remaining 68 haplotypes were singletons. Half of the individuals from Bundala had haplotype 1. Haplotype 4 was common in Bundala and Koggala, while haplotype 2 and 3 were rare in Bundala and Koggala. Haplotype 10 was only found in two individuals from Bundala. The network diagram in Figure 2 shows only one branch with more than three

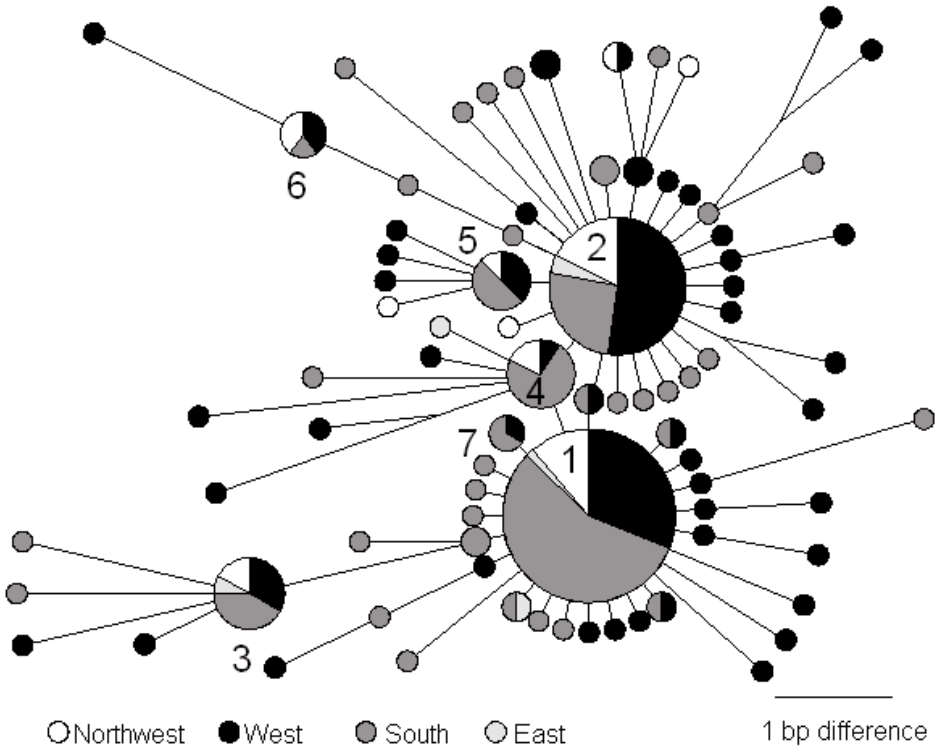

Fig. 2. Median-joining network of COI mtDNA haplotypes in *Fenneropenaeus indicus,* sampled from northwest to east coastal belt of Sri Lanka. Each haplotype is represented by a pie and its area is proportional to its relative frequency (smallest = 1); length of lines from the centre of the pie reflects the number of nucleotide differences between haplotypes.

mutations between two haplotypes and has six branches with two mutations between two haplotypes. Thus, we can conclude that most of the existing intermediate haplotypes have been reasonably sampled in this study.

Considerable genetic diversity was detected in all samples (Table 2). Both the overall nucleotide diversity ($\pi = 0.035$) and the overall mean haplotype diversity ($h = 0.876$) were high. The haplotype diversity was highest at Negombo (0.95) and declined towards Bundala in southeast (0.74). No patterns were observed with nucleotide diversity, except that Bundala was the least variable (Table 2). The values reported for h and π from Trincomalee are within the range reported for all the other samples, despite the smaller sample size from this site.

Population genetic structure

The spatial analysis of molecular variance (SAMOVA) revealed clustering of two separate groups: one with the Bundala and Koggala samples, and the other with the samples from Kalpitiya, Rakawa, Hendala, Negombo coast and Negombo lagoon. The $\Phi_{st} = 0.022$ was significantly different between these two groups ($p = 0.048 \pm 0.007$), while the percentage variance values among populations within the groups were ~ 0 ($\Phi_{st} = 0.0$, $p = 0.844 \pm 0.011$). Analyses based on three groups gave lower subdivision among groups, but also a lower P-value. Pairwise differences (F_{st} and Φ_{st}) among samples were small (Table 3), but agreed well with the SAMOVA result (Fig. 3): with Bundala and to some extent Koggala

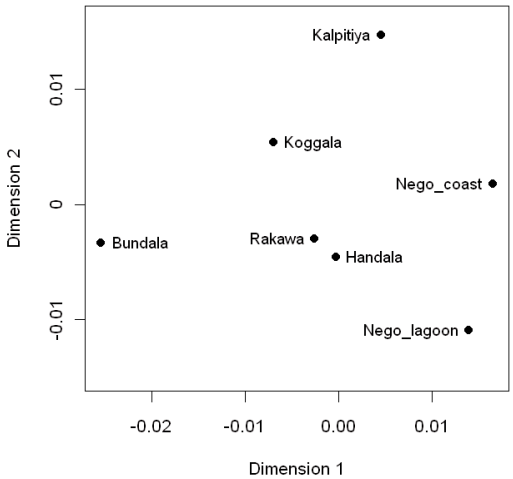

Fig. 3. Multidimensional scaling analysis of pairwise genetic distances (Φ_{st}) between seven samples of *F. indicus* along the coast of Sri Lanka.

deviating from the others. Out of 21 pairwise F_{st} comparisons, the three populations from Hendala, Negombo coast and Negombo lagoon were nominally significantly different from the Bundala population (Table 3). Similarly, Φ_{st} comparisons showed that the Negombo coast and Negambo lagoon populations were nominally different from the Bundala population. All comparisons were non-significant after applying the Bonferroni correction ($p > 0.0025$), except the F_{st} obtained for

Table 2. Diversity in *Fenneropenaeus indicus*, by sampling site, region, and for all data combined. For each sampling site, the following data are presented: number of samples (N), number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π), estimates of time since expansion obtained from the mismatch distribution (τ), and the corresponding time in thousand years (t).

Region/ locality	N	N_h	h	π (×100)	τ	t
East						
Trincomalee	5	5	0.90 ± 0.126	3.95 ± 0.03	–	–
Southern						
Bundala	28	11	0.74 ± 0.084	2.32 ± 0.01	0.61	23–36
Rakawa	46	19	0.83 ± 0.050	3.51 ± 0.02	3.54	134–210
Koggala	25	16	0.90 ± 0.054	3.13 ± 0.02	2.59	98–154
Western						
Hendala	29	16	0.88 ± 0.047	3.72 ± 0.03	3.45	130–205
Negombo-lagoon	32	21	0.95 ± 0.024	4.62 ± 0.03	3.61	136–214
Negombo-coast	31	22	0.95 ± 0.031	3.68 ± 0.03	2.88	109–171
Northwest						
Kalpitiya	21	8	0.82 ± 0.065	2.76 ± 0.02	2.55	96–151
All	217	82	0.88 ± 0.019	3.47 ± 0.02	3.13	118–186

Bundala and Negombo lagoon. It should, however, be noted that as the correction assumes that tests were independent and is, therefore, too conservative for the comparisons made.

Variation in the F_{st} was independent of geographical distances (Mantel test $p = 0.118$, 1000 permutations), although the genetic distances did show some geographic structuring. The estimated F_{st} values, including comparisons with Bundala, which had shown indication of divergence from the other samples, showed a significant correlation with geographical distance to the other sampling locations (Fig. 4a). A clearer pattern was observed with the Φ_{st} values, which were positively correlated with geographical distance, (Mantel test, $p = 0.025$, 1000 permutations); this is mainly apparent when samples were separated by more than ~130 km (Fig. 4b).

Demographic history

The mismatch analysis produced unimodal distributions for all samples separately and for all samples combined (Fig. 5). All distributions followed the distribution expected under the sudden expansion model, with p ranging from 0.07 to 0.67. The τ-values ranged from 2.1 to 3.5 for all populations except Bundala, suggesting an ancient population expansion around 100 to 200 thousand years ago. The sample at Bundala had the lowest τ-value (0.61), corresponding to a population expansion 23 to 36 thousand years ago (Table 2).

In all populations, Tajima's D statistic was negative, and significant (p ranging from 0.002 to 0.04) indicating either sudden expansion (Aris-Brosou and Excoffier 1996) or recovery from selection. Chakraborty's test and Fu's Fs were all significant for the region-wise analysis ($p < 0.001$), further supporting the observed imbalance.

Discussion

The main finding of this study is a high variation in the mtDNA COI region in *F. indicus,* pointing to the presence of a large population with signs of isolation by distance along the coast.

A high level of genetic diversity seems to be characteristic of many decapods (McMillen-Jackson 2004a), although it reflects effective population size, which may vary among different geographical regions throughout the species range due to historical population dynamics. The nucleotide diversity values (π) reported in this study are in the same range as those derived from mtDNA variation of tiger prawn (*P. monodon*) (Klinbunga et al. 1999; Benzie et al. 2002) brown shrimp (*F. aztecus*), *P. vannamei* (Valles-Jimenez et al. 2006), *F. duorarum* (McMillen-Jackson and Bert 2004a), *L. setiferus* (McMillen-Jackson and Bert 2003) and *F. chinensis* (Kong et al. 2010) populations; although Cui et al. (2007), in contrast, reported relatively low haplotype diversity value (0.24) in *P. chinensis*. The nucleotide diversity observed in this study is interestingly close to the highest level of π observed for *Penaeus monodon* in Indonesia, which could be explained by migrations from the oceans to the east and west of the studied region (Benzie et al. 2002). The high variation observed in our study does not appear to stem from admixture of two separate lineages, but rather from a large population that has been expanding from the onset of the last glacial ice age period, known to have caused wide range sea level fluctuations, or perhaps even earlier (Voris 2000). Only the population in Bundala shows a different expansion, which may have occurred around the last glacial maximum, 21000 years ago. Studies of species living at higher latitudes, such as *Crangon crangon* in the North Atlantic (Luttikhuizen et al. 2008) and *Aristeus antennatus* in the Mediterranean (Roldan et al. 2009), reported much lower nucleotide (one tenth or less) diversity (π).

The present study gives an indication of a historic period of sudden population expansion in *F. indicus.* A sudden population expansion may result in an excess of rare haplotypes, as more haplotypes are produced by mutation than are removed by genetic drift. Alternatively, the same patterns could have resulted from a recovery of variation following selective sweep for a favorable haplotype, with new haplotypes being

Table 3. Pairwise genetic distances between *F. indicus* populations in the waters along the coast of Sri Lanka, based on the mitochondrial DNA COI gene haplotypic diversity. F_{st} (×100) values are shown above the diagonal, Φ_{st}(×100) are below it. Negative estimates of genetic distances were small and were rounded up to zero.

Region	South			West			Northwest
	Koggala	Bundala	Rakawa	Hendala	Negombo coast	Negombo lagoon	Kalpitiya
Koggala	–	3.75	0	0.07	0	0.84	0
Bundala	0	–	1.26	3.12*	3.85*	6.06**	0.51
Rakawa	0	0.25	–	0.04	1	1.16	0
Hendala	0	1.68	0	–		0	0
Negombo coast	1.45	4.23*	0.07		–		0.21
Negombo lagoon	2.55 [a]	3.88*	0.41			–	0.24
Kalpitiya	1.40	3.41	0				–

Statistical significance levels: p = 0.1-0.05: *a*; p = 0.05-0.01: *; p = 0.01-0.001: **.

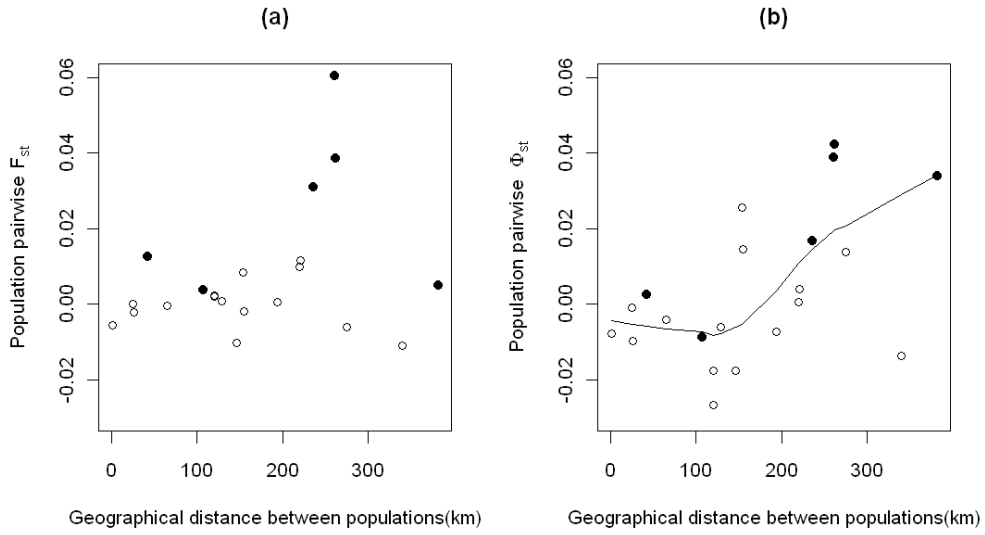

Fig. 4. Relationship between pairwise genetic and geographic distances constructed with F_{st} values (a) and Φ_{st} values (b) for seven sampling locations of *F. indicus*. Comparisons including the Bundala sample are indicated by filled circles.

generated by mutations, but all studied populations showed a significantly negative Tajima's D value.

Haplotype diversity showed a geographical cline along the coastal belt from west (Negombo lagoon) to south (lowest at Bundala), suggesting that gene flow may be limited or geographically restricted, as gene flows tend to homogenize populations genetically. Random mixing appears to take place within a range of ~130 km, reflecting dispersal distances and population density; at larger distances genetic divergence is observed. Although homogeneity among populations was evident according to the haplotypes, genetic divergences, though marginally significant, were detected both for Φ_{st} and F_{st} between the Bundala population and the populations of the west coast.

While adult *F. indicus* are epibenthic and, as such, should not be greatly influenced by currents, the pelagic larval stage may be more susceptible to dispersal and can be expected to increase gene flow. This may explain the genetic similarities among the sampled locations, although the genetic differentiation may not reflect the population separation if the splitting is recent. It is therefore interesting to observe the divergence of the Bundala population. Being the first population genetic study in Sri Lankan marine waters, no literature exists to compare the pattern observed for *F. indicus* in the Bundala region with that in other marine invertebrates of the area. The exact dispersal potential of *F. indicus* in Sri Lankan waters is also not known. Local dispersal of *F. indicus* (Hayashi et al. 1992), lobsters and crabs (Hayashi 2000; Aoki et al. 2008) has been observed in the region due to oceanographic forces and larval behaviors in tropical and subtropical waters. The highest exchange of individuals probably occurs between geographically adjacent locations (Cowen et al. 2000), as observed in the western and southern regions in this study. Also, in most regions, inter-estuarine dispersal of adult *F. indicus* has rarely been recorded. Low effective population size or lower diversity at Bundala may be the result of several factors, such as recent colonization, the environment and/or isolation by distance, strengthened by ocean currents (Hellberg 2009). The Bundala location does not have long history of shrimp fishing comparison with the west region, where well-established shrimp fisheries have been reported for the last 200 years (Jayawardane and Perera 2003). In order to understand the origin of the Bundala population, continental population structure and variation on the east coast need to be determined.

The genetic patterns observed are likely to be affected by the combination of consistency of spawning period with

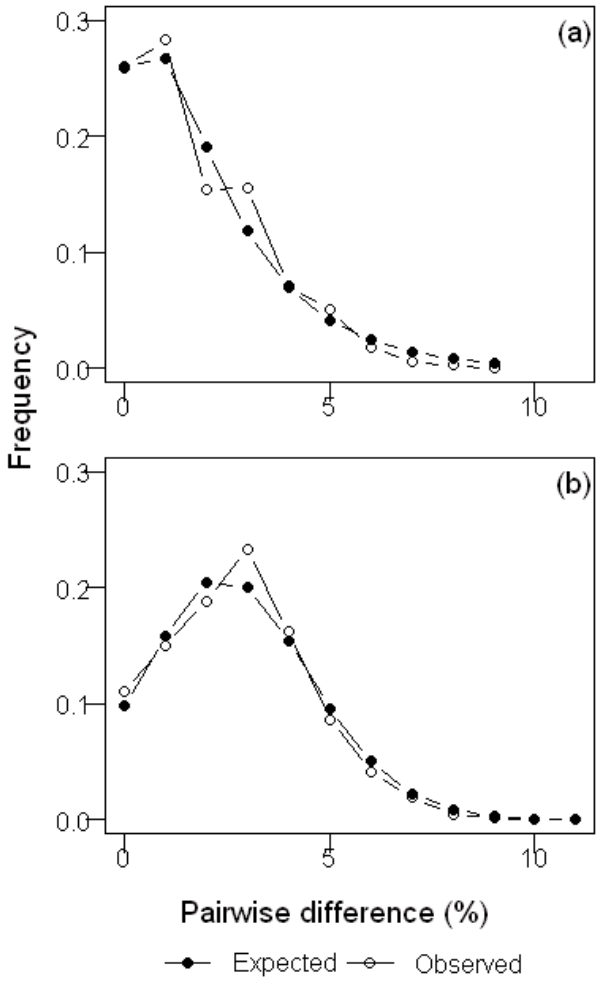

Fig. 5. Distribution of pairwise differences between COI mtDNA sequences of *F. indicus* from Bundala (a), and for all sequences except the Bundala sample (b). Open circles represent the observed distribution. Filled circles represent the sudden expansion model distribution based on the same mean and variances as the observed distribution. The *X*-axes indicate the number of pair wise differences (*i*) between haplotypes, the *Y*-axes the relative frequency of haplotypes with *i* differences (*F*(*i*)).

respect to the presence of monsoonal currents, the directional change in currents over the year (Schott and McCreary 2001) and upwellings (Vinayachandran 2004). Mature bottom-dwelling *F. indicus* have their peak spawning in the coastal waters at the onset of the monsoon period (Jayawardene et al. 2002b), which coincides with the strong southwest monsoonal currents that flow towards the east coast and slightly away from the southeast of the country, as reported by Vinayachandran (2004). A sand dune at the mouth of the Bundala lagoon (Smakhtin and Piyankarage 2003) may also act as a physical barrier that restricts or selects the larvae that enter the lagoon to complete their life cycle, causing a bottleneck effect. It is difficult, however, to make assumptions about the dispersal of crustaceans in the oceans during their larval stage with respect to oceanic currents (Barber et al. 2002). To reveal the scale of larval dispersal, it is important to perform periodic monitoring of larval behavior, larval dispersal, and to take into consideration the present and past history of coastal currents.

The impacts of humans on the observed patterns cannot be excluded. In the 1980s, penaeid shrimp culture trials were made at Rakawa lagoon, taking brooders considered suitable for aquaculture from Kalpitiya and other parts of northwest coast. The trials were unsuccessful and were not documented, but Davenport et al. (1999) reported that one successful *P. monodon* stocking was made at Rakawa. Whether such trials can explain the closer relationships of the Rakawa sample with the west coast than that observed for Koggala is not known, as information is lacking for *F. indicus*. The pattern might equally well reflect some statistical uncertainty due to sampling or random fluctuations.

Patterns in mtDNA of *F. indicus* indicate a large population with limited dispersal in Sri Lankan waters, which may contribute to the observed differences among sampling localities. The conservative view of a large single panmictic breeding stock should be reevaluated. As shown by Carvalho and Hauser (1994), geographically and genetically different populations should be recognised as different stocks and managed separately, with specific management approaches. Similarly, for sustainable management of continuous populations showing signs of isolation by distance, the spatial patterns need to be considered, because although recruitment may occur at distinct locations, mixing may occur between neighboring locations. A further study, assessing variation in other genetic markers may be needed to obtain unequivocal results showing the true pattern, as different genetic markers can tell different stories.

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Supporting information

Table S1: Cytochrome oxidase subunit I (COI) haplotype frequencies for *Fenneropenaeus indicus* at eight locations in Sri Lanka, and Gene Bank accession number.

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