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Genetic Diversity of Three Spotted Seahorse, *Hippocampus trimaculatus* (Leach, 1814) in India Using Four Microsatellite Loci

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Abstract

Seahorse populations are declining year by year not only in India but also throughout the world, because of over-fishing and increasing demand in Chinese market. The three spotted seahorse, *Hippocampus trimaculatus* is one of the dominant species and distributed all along the Indian coast. To study the genetic structure is very essential to conserve these species effectively. *Hippocampus trimaculatus* samples (n = 60/population) were collected from Mullimunai in Palk Bay, Tuticorin in Gulf of Mannar and Vizhinjam in south Malabar in India as by-catch in small trawlnets. Microsatellites are being widely applied in animal genome mapping and phylogenetic analysis because of their co-dominant inheritance and high degree of polymorphism. The molecular polymorphism of microsatellite DNA has proved to be a potent tool in the analysis of several aspects of population genetics. In the present study, four microsatellite primers were used to investigate the genetic difference and structure of three selected populations of *H. trimaculatus*. The result showed the overall F_{ST} value (0.0989) of the microsatellite loci between Mullimunai and Vizhinjam was significantly different ($p<0.0001$). The genetic distance between Mullimunai and Tuticorin was 0.183; between Tuticorin and Vizhinjam was 0.461; and Mullimunai and Vizhinjam was 0.837. There was no statistical evidence of recent severe bottlenecks in any of the three populations. Continuous monitoring of microsatellite variations within the populations of all the three locations was suggested to determine whether genetic variation within the populations is stabilized between year classes.

Keywords: *Hippocampus trimaculatus*, genetic diversity, microsatellite, molecular marker

Introduction

A genetically inherited variant in which the phenotype can be inferred from the genotype during genetic screening is known as genetic marker. The utility of DNA-based marker is generally determined by the technology that is used to reveal DNA- based polymorphism (Gupta and Gupta, 2011). They are also used to identify different species in the event of taxonomic disputes and also to detect genetic diversity in a species. (Gopalakrishnan and Mohindra, 2001). Microsatellites are short tandemly arrayed di-, tri-, or tetra- nucleotide repeat sequences with repeat size of 1-6 bp repeated several times flanked by regions of non-repetitive unique DNA sequences (Tautz, 1989). The development of polymorphic microsatellite markers to determine the population structure of the Patagonian toothfish, *Dissostichus eleginoides*, has been reported by Reilly and Ward (1998). Microsatellites that have been largely utilized for population studies are single locus ones in which both the alleles in a heterozygote show co-dominant pattern of inheritance (Gopalakrishnan and Mohindra, 2001). Many authors have reported microsatellite polymorphism and sequences in some marine and freshwater fish species using population genetic analysis (Appleyard *et al.,* 2002; Han *et al.,* 2000). Neff *et al.* (1999) described 10 microsatellite loci from the bluegill (*Lepomis macrochirus*) and discussed their evolution within the family Centarchidae. Kellog *et al.* (1995) applied microsatellite-fingerprinting approach to address questions about paternity in cichlids.

A number of attempts have been made to study the cross- species amplification of microsatellite loci in fishes. May *et al.* (1997) reported the microsatellite genetic variation through cross species amplification in sturgeons *Acipenser* and *Scaphirhynchus*. Only two studies were made for population genetics in seahorses using microsatellite marker in worldwide so far. Four polymorphic microsatellite loci were used to asses biological parentage from a natural population of the western Australian seahorse *Hippocampus angustus* (Jones *et al.,* 1998). Later, Jones *et al.* (2003) used three microsatellite loci for genetic parentage in *Hippocampus subelongatus.* There is no such molecu-

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lar study was reported in *H. trimaculatus* in India as well as in other countries.

Seahorse populations are declining year by year not only in India but throughout the world, because of overfishing and increasing demand in Chinese market (Lipton and Thangaraj, 2002). Among the six Indian seahorse species *viz*., *H. kuda, H. fuscus, H. histix, H. kelloggi, H. trimaculatus* and *H. spinossisimus*, the three spotted seahorse, *H. trimaculatus* is the dominant species and distributed throughout entire coast of the nation (Murugan *et al.,* 2008). The body colour of this species is golden orange, sandy coloured to totally black, some have striped in brown and white colour. The distinguish characters are: very sharp hook like cheek and eye spine, quite flat in appearance, narrow head, no nose spine, large dark spots on the dorso-lateral surface of the first, fourth and seventh trunk rings more common in male than female. They inhabited more than 20 m deep waters with gravel, sandy or clay bottom. To conserve these species effectively, it is essential to study the genetic structure of it. In the present study, the genetic structure of three populations was assessed using microsatellite markers.

Materials and methods

Collection of samples

The three spotted seahorse, *Hippocampus trimaculatus* (Fig. 1) samples (n = 60/population) were collected from Mullimunai (9° 44' N; 79° 10' E) in Palk Bay, Tuticorin (8° 47' N; 78° 80' E) in Gulf of Mannar and Vizhinjam (8° 21' N; 76° 58' E) in south Malabar (Fig. 2) as by-catch in small trawlnets. The tissue samples (finclips) for DNA extraction were stored in sterile eppendorf tubes containing 95% ethanol.

Fig 1. The male three spotted seahorse, *H. trimaculatus*

DNA extraction and microsatellite DNA analysis

Total DNA was extracted by standard phenol-chloroform method (Taggart *et al.,* 1992). The DNA samples were screened for DNA variation at four microsatellite loci: *Han 05, Han 06, Han 15* (Jones *et al.,* 1998), *Han 16* (Jones *et al.,* 2003). Polymerase chain reactions (PCR) were carried out in a thermal cycler (M.J. Research, USA) employing the microsatellite primers. Amplifications were performed in 25 µl reaction mixture containing 1x reaction buffer (10 mM Tris, 50 mM KCl, 0.01% gelatin, pH 9.0) with 1.5 mM $MgCl_2$, 5 pM of each primer, 200 mM dNTPs, 2 U Taq DNA polymerase (Genei, India) and 20 ng of template DNA. The temperature profile of the PCR was; 94°C for 3 min of an initial denaturing cycle followed by 35 cycles of 94°C denaturation for 30 s, a 50-54°C annealing for 30 s (varied according to primers), a 72°C extension cycle for 1 min; followed by a final extension step at 72°C for 5 min. Then each PCR product (2 µl) was electrophoresed in 10% acrylamide gel, which was fixed and stained with silver nitrate.

Data analysis

To analyze the variation in microsatellite loci, allele frequencies at each locus were calculated with Genetix software (Belkhir *et al.,* 1997). A locus was considered to be polymorphic when the frequency of the most common allele was equal to or less than 0.99 (Nei, 1987). The mean number of alleles per locus, observed and expected heterozygosities (H_{obs} and H_{exp}) and percentage of polymorphic loci for overall and each population were calculated with Genetix software.

Exact P-tests for conformity to Hardy-Weinberg Equilibrium (probability and score test) were performed by the Markov Chain method using Genepop (Raymond and Rousset, 1998) with parameters, dememorization = 1000; batches = 10 and iterations = 100; and based upon a null hypothesis of random union of gametes. The significant criteria were adjusted for the number of simultaneous tests using sequential Bonferroni technique (Rice, 1989).

The coefficient of genetic differentiation (F_{ST}) and the inbreeding coefficient (F_{IS}) were estimated through estimator of Weir and Cockerham (1984). Estimation of average F_{ST} and determining whether the values are significantly

Fig 2. Map showing the collection site

different from zero; and calculation of pair-wise population F_{ST} values (θ) and their significance levels, were carried out using Genepop. This program performs numerical resampling by bootstrapping (1000 times) and jack-knife procedures in order to estimate confidence intervals and the significance of values. The F_{ST} value is used to estimate the amount of gene flow (Nm) using the corrected private - allele-based model of Barton and Slatkin (1986) in Genepop, between populations. The expected frequency of null alleles was calculated according to Van Oosterhoust *et al.* (2004, 2006) using Microchecker.

The three populations were tested for genetic bottleneck using the software Bottleneck to determine whether the populations exhibited the observed gene diversity higher than the expected equilibrium gene diversity by Wilcoxon sign-rank test (Luikart, 1996). Genetic similarity and distance between pairs of populations of *H. trimaculatus* were estimated using Popgene (Yeh *et al.*, 1999). Nei and Li's (1979) pair-wise genetic similarity (SI) among the three populations were computed and converted by Popgene into genetic distance (GD) according to Hillis and Moritz's (1990) formula, GD = 1- SI.

Results

All the four amplified microsatellite loci were polymorphic in all the three populations. All the four primers indicated positive F_{IS} values in different populations (Tab. 1). The estimated null allele frequency was not significant ($p < 0.05$) at all the four tested loci using different algorithms, indicating the absence of null alleles and false homozygotes.

There was no significant association indicative of linkage disequilibrium ($p > 0.05$) between any pair of microsatellite loci for any population. It was therefore assumed that allelic variation at microsatellite loci could be considered independent. The F Statistics and gene flow (Nm) for each locus in the overall populations are given in Tab. 2. In adittion genetic variations such as observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) in the overall populations for each locus are given in Tab. 2. The summary of genetic variations including inbreeding coefficient (F_{IS}), probability value of significant deviation from Hardy Weinberg Equilibrium (P_{HW}), probability value of significant heterozygosity deficiency (P_{score}) are given in Tab. 1.

Tab. 1. Summary of genetic variation statistics of four microsatellite loci in three populations

Location Locus		Mullimunai	Tuticorin	Vizhinjam
Han-05	H_{obs}	0.2000	0.3000	0.2000
	H_{nb}	0.7038	0.7385	0.6564
	F_{IS}	0.7210	0.6000	0.7010
	P_{HW}	0.0000	0.0000	0.0000
	P_{score}	0.0000	0.0000	0.0088
Han-06	H_{obs}	0.0000	0.0500	0.1000
	H_{nb}	0.9179	0.8910	0.9128
	F_{IS}	1.0000	0.9450	0.8930
	P_{HW}	0.0000	0.0000	0.0000
	P_{score}	0.0000	0.0000	0.0000
Han-15	H_{obs}	0.0000	0.0000	0.0000
	H_{nb}	0.7436	0.8051	0.7385
	F_{IS}	1.0000	1.0000	1.0000
	P_{HW}	0.0000	0.0000	0.0000
	P_{score}	0.0000	0.0000	0.0000
Han-16	H_{obs}	0.1000	0.3000	0.1500
	H_{nb}	0.9231	0.9410	0.8628
	F_{IS}	0.8340	0.6870	0.8300
	P_{HW}	0.0000	0.0000	0.0000
	P_{score}	0.0000	0.0000	0.0000
Mean Overall Loci	H_{obs}	0.0750	0.1625	0.1125
	H_{nb}	0.8221	0.8439	0.7926
	F_{IS}	---	---	---
	$P_{(0.95)}$	1.0000	1.0000	1.0000
	$P_{(0.99)}$	1.0000	1.0000	1.0000
	A_n	8.5000	9.2500	7.0000

H_{obs} = Observed heterozygosity; H_{nb}= Heterozygosity (Non-biased) for small populations; F_{IS}=Inbreeding coefficient; P_{HW} = Probability value of significant deviation from HWE; P_{score} = Probability value of significant heterozygosity deficiency; $P_{(0.95)}$ = Polymorphism at 0.95 criteria; $P_{(0.99)}$ = Polymorphism at 0.99 criteria; A_n = Mean number of alleles per locus

Tab. 2. F statistics and Observed (H_{obs}) and Expected (H_{exp}) heterozygosity of overall populations

Locus	F_{ST}	Nm	H_{obs}	H_{exp}	Ave. Het.
Han-05	0.08637	2.8031	0.2333	0.7492	0.6821
Han-06	0.01258	5.8539	0.0500	0.9301	0.8846
Han-15	0.06518	2.1581	0.0000	0.8364	0.7433
Han-16	0.03938	4.3581	0.1833	0.9450	0.8862
Mean ± SD	0.05088 ± 0.03195	3.7933 ± 1.6552	0.1097 ± 0.1097	0.0910 ± 0.0910	0.1028 ± 0.1028

Tab. 3. The Infinite Allele Model (IAM) and Two Phase Model (TPM) of mutations for four microsatellite loci in three *H. trimaculatus* populations

Population		IAM			TPM	
	Locus	He	Heq	P	Heq	P
Mullimunai	Han-05	0.704	0.507	0.0460	0.611	0.1670
	Han-06	0.918	0.870	0.0460	0.904	0.2690
	Han-15	0.744	0.501	0.0130	0.599	0.0480
	Han-16	0.923	0.870	0.0220	0.904	0.1440
Tuticorin	Han-05	0.738	0.589	0.1010	0.685	0.3060
	Han-06	0.891	0.812	0.0180	0.862	0.1400
	Han-15	0.805	0.650	0.0380	0.744	0.1380
	Han-16	0.941	0.908	0.0230	0.929	0.2240
Vizhinjam	Han-05	0.656	0.504	0.1720	0.606	0.3840
	Han-06	0.913	0.854	0.0260	0.892	0.1570
	Han-15	0.738	0.583	0.0970	0.687	0.3050
	Han-16	0.863	0.701	0.0010	0.784	0.0120

The probability test revealed that the observed allele frequencies in most of the loci showed significant deviation ($p < 0.05$) from Hardy Weinberg Equilibrium (Tab. 1). The Infinite Allele Model (IAM), Two Phase Model (TPM) and the significance test for each locus in the three populations are given in Tab. 3. The test did not exhibit significant excess of observed gene diversity ($p > 0.05$) on average loci for all the locations under both mutation models. There was no indication of reduction of rare alleles, indicating the absence of evidence of recent genetic bottleneck in any of the populations.

The coefficient of genetic differentiation (pair-wise F_{ST}) differed between populations significantly ($p < 0.0001$) and the result is given in Tab. 4. Nei's (1978) unbiased genetic distances estimated between pairs of three populations of *H. trimaculatus* are given in Tab. 5. The genetic distance between Mullimunai and Tuticorin was 0.183; between Tuticorin and Vizhinjam was 0.461; and Mullimunai and Vizhinjam was 0.837.

Tab. 4. Fisher's pair wise F_{ST} values in three populations (above diagonal) and their significant level (below diagonal)

Populations	Mullimunai	Tuticorin	Vizhinjam
Mullimunai	-----	0.01096	0.09895
Tuticorin	NS	-----	0.05682
Vizhinjam	$p<0.0001$	NS	----

Discussion

Generally, the development of new species-specific microsatellite primers is expensive and time consuming, but cross-species amplification is cheap and fast. Primers developed for a particular species have been successfully tested for cross-species amplification in several fish species (Gopalakrishnan *et al.,* 2004; Lal *et al.,* 2003; Mohindra *et al.,* 2000). In the present study, successful cross-priming was obtained with four primer pairs and all the loci were polymorphic and ideal to be used as markers in stock identification studies. However, the optimum annealing temperature to get scorable band in *H. trimaculatus* slightly differed from the resource species. The present study demonstrated successful cross-priming of microsatellite loci, between the fish species of closely related. The use of heterologous PCR primers would significantly reduce the cost of developing similar set of markers for other *Hippocampus* species found in India.

The number of alleles at different microsatellite loci in *H. trimaculatus* varied from 6 to 26 with an average value of 15. Primers *Han 05* and *Han 16* exhibited maximum

Tab. 5. Nei's (1978) Genetic distance between populations

Populations	Mullimunai	Tuticorin	Vizhinjam
Mullimunai	****		
Tuticorin	0.183	****	
Vizhinjam	0.837	0.461	****

number of allele, meaning 22 and 26 respectively compared to other primers (six alleles). High microsatellite allele variation was recorded in Thai silver barb (*Puntius gonionotus*) in four microsatellite loci with average of 13.8 alleles per locus (Kamonrat, 1996), and in a number of marine fishes such as the whiting (14-23 alleles/locus) (Rico *et al*., 1997) and red seabream (16-32 alleles/locus) (Takagi *et al.,* 1999). Jones *et al.* (1998) reported 9 to 22 alleles in the Australian seahorse, *H. angustus* by using four microsatellite loci primers. Neff and Gross (2001) reported that marine species had greater microsatellite allele variation as compared to freshwater species and that this was consistent with the increased effective population sizes.

Variations of allele sizes were quite low for *Han-15* locus which might be due to their small number of repeat units, and similar level of allele size variation is reported in selected freshwater teleosts (Carvalho and Hauser, 1994). The same kind of relationship was observed in *H. angustus* (Jones *et al.,* 1998) where the allelic size of locus *Han-15* was somewhat less with less number of repeat ($[GT]_9$). However, the locus, *Han-06* has 22 alleles in the present study and this is comparable with the earlier reports of 22 alleles in the Australian seahorse (Jones and Avise, 2001). The mean observed number of alleles at each locus (na = 15.00) in the present study was similar as in *H. angustus* (na = 15.75) (Jones *et al.,* 1998).

In *H. trimaculatus,* the mean observed heterozygosity (H_{obs}) for locus *Han-05* per population was 0.2333 and the mean expected heterozygosity (H_{exp}) per locus per population was 0.7492. In the case of *H. angustus*, Jones *et al*. (1998)*,* reported a higher value of mean observed heterozygosity (H_{obs} = 0.9490) for the same locus. However, the mean expected heterozygosity (H_{exp}) was much higher for *Han-06* in the present study (H_{exp} = 0.9301), but in *H. angustus* it was less (H_{exp} = 0.08770) (Jones *et al.,* 1998). In *H. trimaculatus*, a significant overall deficiency of heterozygosity was revealed in all the populations for all the loci whereas in the *H. angustus* all the loci showed significant by high value of heterozygotes (Jones *et al.,* 1998).

A significant deviation from HWE were found within samples across loci rather than within loci and across most of the samples. There was also no instance of non-amplifying samples in repeated trials with any of the primer pairs in *H. trimaculatus*. Van Oosterhout *et al.* (2004) suggested that, the overall homozygosity can be due to deviations from panmixia, inbreeding, short allele dominance, stuttering or large allele dropouts.

Seahorses are generally characterized by sparse distribution, low mobility, small home ranges, low fecundity, mate and habitat fidelity. Due to this, inbreeding can happen, which will result in the deficiency of heterozygotes and deviation from HWE. Similar situation was reported in other fishes that showed decline in catches due to over exploitation (Gopalakrishnan *et al.,* 2004; Rico *et al.,* 1997, Yue *et al.,* 2000).

There were twelve stock specific alleles found in Mullimunai, thirteen in Tuticorin and nine alleles in Vizhinjam population. The detection of these private alleles in specific populations might be the absence of mixing of the gene pool between populations. In *Clarias macrocephalus*, Na-Nakorn *et al.* (1999) reported twenty stock-specific markers in three loci in four populations in Thailand. Scribner *et al.* (1996) reported 22 stock specific alleles in three populations of Chinook salmon (*Oncorhynchus tshawytscha)* in Canada.

The overall F_{ST} value (0.0989) of the microsatellite loci between Mullimunai and Vizhinjam was significantly different ($p < 0.0001$). Levels of genetic differentiation demonstrated here for *H. trimaculatus* between the Mullimunai and Vizhinjam population (F_{ST} = 0.0989) are comparable to those significant values seen in the Pacific herring (F_{ST} = 0.023), Atlantic herring (F_{ST} = 0.035) and widespread anadromous fish like Atlantic salmon (F_{ST} = 0.054) (McConnell *et al.,* 1995). In the present study, the Nm value indicated chances of restricted migration between populations and that genetic differentiation over time might have occurred due to genetic drift. However, low values of Nm have been reported in species exhibiting significant genetic differences and geographic isolation among populations as in the present study (Angers *et al.,* 1995; Coughlan *et al.,* 1998).

A significant reduction in microsatellite heterozygosity, but non-significant loss of microsatellite alleles was observed in all the three populations. Comparison of observed heterozygocities with that expected, under a two phase model (TPM) did not support the hypothesis of a severe bottleneck in the seahorse populations. Therefore, there was no statistical evidence of recent severe bottlenecks in any of the three populations. Continuous monitoring of microsatellite variations within the populations of all the three locations is suggested to determine whether genetic variation within the populations is stabilized between year classes.

Conclusions

The analysis using novel hypervariable microsatellite loci in *H. trimaculatus* revealed several significant results. First, the potential use of heterologous PCR primers was explored and they appeared to be conserved in this species. Second, all the four microsatellite loci were polymorphic and showed heterogeneity in allele frequency in three populations. Third, the study suggested that these three natural populations of this species, *viz.,* Mullimunai, Tuticorin and Vizhinjam are divergent in their genetic characteristics can be clearly identified through microsatellite loci.

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References

Angers B, Bernatchez L, Angers A, Desgroseillers L (1995). Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J Fish Biol 47:177-185.

Appleyard SA, Ward RD, Grewe PM (2002). Genetic stock structure of big eye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. J Fish Biol 60:767-770.

Barton NH, Slatkin M (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity 56:409-415.

Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1997). GENETIX logicielsous windows pour Ia ge'ne'tique des populations, http://www.univ- montp2.fr/~gentix/genetix/html.

Carvalho GR, Hauser I (1994). Molecular genetics and the stock concept in fisheries. Rev Fish Biol Fish 4:326-350.

Coughlan J, Imsland AK, Galvin PT, Fitzgerald RD, Naevdal G, Cross TF (1998). Microsatellite DNA variation in wild populations and farmed strains of turbot from Ireland and Norway: a preliminary study. J Fish Biol 52:916-922.

Gopalakrishnan A, Mohindra V (2001). Molecular markers, A22-A27. In: Mohindra V, Lal KK, Gopalakrishnan A, Ponniah AG (Eds.). Molecular markers: tools for fish population genetic analysis protocols, NBFGR- NATP, Lucknow, U.P. India.

Gopalakrishnan A, Musammilu KK, Muneer PMA, Lal KK, Kapoor D, Ponniah AG, Mohindra V (2004). Microsatellite DNA markers to assess population structure of red tailed barb, *Gonoproktopterus curmuca*. Acta Zool Sin 50(4):686-690.

Gupta A, Gupta N (2011). PCR-RAPD and cross species rat microsatellite loci amplification in *Mastomys* (*Praomys*) *caucha* Smith, 1936. Indian J Biotech 10(1):45-51.

Han K, Li L, Leclerc GM, Hays AM, Ely B (2000). Isolation and characterization of microsatellite loci for striped Bass (*Morone saxatilis*). Mar Biotech 2:405-408.

Hillis DM, Moritz C (1990). Molecular systematics. Sinauer Assoc. Inc., Sunderland, U.S.A.

Jones AG, Avis JC (2001). Mating systems and sexual selection in male pregnant pipefishes and seahorses: Insights from microsatellite- based studies of maternity. J Heredity 92(2):150-158.

Jones AG, Kwarnemo C, Moore GI, Simmons SI, Avis JC (1998). Microsatellite evidence for monogamy and sex biased recombination in the western Australian seahorse *Hippocampus angustus*. Mol Evol 7(11):1497-1505.

Jones AG, Moore GI, Kvarnemo C, Walker D, Avis JC (2003). Sympatric speciation as a consequence of male pregnancy in seahorses. Proc Nat Acad Sci USA 100:6598-6603.

Kamonrat W (1996). Spatial genetic structure of Thai silver barb *Puntius gonionotus* (Bleeker) populations in Thailand. Ph. D. thesis, Delhousie University, Delhousie. Canada, 11-93 p.

Kellog KA, Markert JA, Stauffer JR, Kocher TD (1995). Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. Proc Royal Soc Lond 260(B):79-84.

Lal KK, Singh RK, Mohindra V, Singh B, Ponniah AG (2003). Genetic makeup of exotic catfish *Clarias gariepinus* in India. Asian Fish Sci 16:229-234.

Lipton AP, Thangaraj M (2002). Present status of seahorse fishing along the Palk Bay coast of Tamilnadu. Marine Fisheries Information Service Technical and Extension Series 174:5-8.

Luikart G (1997). Usefulness of molecular markers for detecting population bottlenecks and monitoring genetic change. Ph. D. thesis. University of Montana, Missoula, USA.

May B, Kruger CC, Kincaid HL (1997). Genetic variation at microsatellite loci in sturgeon: primer sequence homology in Acipenser and Scaphirhynchus. Can J Fish Aqua Sci 51:1542-1547.

McConnell SK, O'Reilly P, Hamilton L, Wright JM, Bentzen P (1995). Polymorphic microsatellite markers loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Can J Fish Aqua Sci 52:1862-1873.

Mohindra V, Mishra A, Palanichamy M, Ponniah AG (2001). Cross-species amplification of *Catla catla* microsatellite locus in *Labeo rohita*. Indian J Fish 48(1):103-108.

Murugan A, Dhanya S, Rajagopal S, Balasubramanian T (2008). Seahorses and pipefishes of the Tamilnadu coast. Curr Sci 95(2):253-260.

Na-Nakorn U, Taniguchi N, Nugroho E, Seki S, Kamonrat W (1999). Isolation and characterization of microsatellite loci of *Clarias macrocephalus* and their application to genetic diversity study. Fish Sci 65(4):520-526.

Neff BD, Gross MR (2001). Microsatellite evolution in vertebrates: inference from AC dinucleotide repeates. Evolution 55(9):1717-1733.

Neff BD, Fu P, Gross MR (1999). Microsatellite evolution in sunfish (Centrarchidae). Can J Fish Aquat Sci 56:1198-1205.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei M (1987). Molecular evolutionary genetics. Columbia University Press, New York.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Nat Acad Sci USA 76:5269-5273.

Raymond M, Rousset F (1998). GENEPOP (ver. 3.1): A population genetics software for exact test and ecumenicism. J Heredity 86:248-249.

Reilly A, Ward RD (1998). Microsatellite loci to determine population structure of the Patagonian toothfish, *Dissoslichus eleginoides*. Mol Ecol 8:1753-1768.

Rice WR (1989). Analyzing tables of statistical tests. Evolution 43: 223-225.

Rico C, Ibrahim KM, Hewitt GM (1997). Stock composition in North Atlantic populations of whiting using microsatellite markers. J Fish Biol 51:462-475.

Scribner KT, Gust JR, Fields RL (1996). Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can J Fish Aqua Sci 53:833-841.

Taggart JB, Hynes RA, Prodohal PA, Ferguson A (1992). A simplified protocol for routine total DNA isolation from salmonid fishes. J Fish Biol 40:963-965.

Takagi M, Okamura T, Chow S, Taniguchi N (1999). PCR primers for microsatellite loci in Tuna species of the genus *Thunnus* and its application for population genetic study. Fish Sci 65(4):571-576.

Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Res 17:6463-6471.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Van Oosterhout C, Weetman D, Hutchinson WF (2006). Estimation and adjustment of microsatellite null alleles in non equilibrium populations. Mol Ecol Notes 6:255-256.

Weir BS, Cockerham CC (1984). Estimating *F*-statistics for the analysis of population structure. Evolution 38:1359-70.

Wright S (1978). Evolution and the Genetics of populations. Vol.4. Variability within and among natural populations. University of Chicago Press, Chicago, Illinois.

Yeh FC, Yang RC, Boyle T (1999). POPGENE 32 – Version 1.31. Population genetics software. Hyperlink http.//www.ualberta.ca/~fyeh/fyeh/.

Yue GH, Chen F, Orban L (2000). Rapid isolation and characterisation of microsatellites from the genome of Asian arowana (*Scleropages formosus*, Osteoglossidae, Pisces). Mol Ecol 9(7):1007-1009.