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ONLINE RESOURCES

Development and characterization of eighty-one microsatellite markers in Indian white shrimp, *Fenneropenaeus indicus*, through cross-amplification

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Introduction

Indian white shrimp, *Fenneropenaeus indicus*, is an important crustacean species in the commercial fish landings of southwest and southeast coasts of India. It also forms a major fishery in African coast (Mozambique, Tanzania and Kenya), Sri Lanka, Red Sea and Persian Gulf. To reveal the genetic stock structure and gene mapping studies of *F. indicus*, we developed 81 polymorphic microsatellites through cross-amplification after screening 396 primer pairs from other penaeids. This genetic information will be of immense use in management of stocks and selective breeding programmes of *F. indicus*.

The wild populations of *F. indicus* from different parts of the world may be distinct with respect to phenotypic traits such as growth, fecundity, feed conversion efficiency, salinity tolerance and disease resistance. Determination of genetic variation in natural populations of commercially important fishes would help in identifying their genetic strains, if any. In *F. indicus*, information of the same can be used for its genetic upgradation, fisheries management and conservation programmes. Molecular genetic markers like microsatellites are useful in studying the genetic variability of natural populations (at intraspecific level). Greater the number of microsatellite markers available easier it is to construct the linkage map of a species which would help the breeders to tag the desired genes and consequently breed cultured shrimps. This, points to the need to develop more microsatellite markers for different species.

In the present study, we developed 81 microsatellite markers through cross-species amplification from related species which can be helpful in unraveling the genetic structure among the wild stocks of *F. indicus*.

Materials and methods

For cross-species amplification, altogether a total of 396 primer pairs were identified from different penaeids from published papers (Xu *et al.* 1999; Wang *et al.* 2005; Dong *et al.* 2006; Zhi-Ying *et al.* 2006; Freitas *et al.* 2007; Gao *et al.* 2008; etc.) and from NCBI GenBank accessions (http://www.ncbi.nlm.nih.gov). The cross-species amplification trials were done for 20 to 30 specimens of *F. indicus* in different size groups (100–200 mm in total length), collected from the trawl landings at fisheries harbour, Cochin during September–December 2010. After recording the total length, carapace length, total weight and sex of the specimens, the total DNA was extracted from the gills of the samples, following salting out procedure of Miller *et al.* (1988). Amplifications were performed in Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Carlsbad, USA) using standardized protocols. PCR reactions were carried out in 25 μL reaction mixture containing 1× reaction buffer (10 mM Tris, 50 mM KCl, 0.01% gelatin and pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bengaluru, India), 5 pmol of each primer, 200 mM dNTPs, 2 U *Taq* DNA polymerase (Fermentas, Burlington, Canada) and 25–50 ng of template DNA. The reaction mixture was preheated at 94°C for 5 min followed by 25 cycles (94°C for 30 s, annealing temperature depending upon the T_m value of primer (usually 50–60°C, see table 1) and 72°C for 2 min). The reaction conditions were standardized for different primers for fine results. The amplified products were electrophoretically analysed through 10% nondenaturing

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Table 1. Characteristics of 81 microsatellite loci in *F. indicus* developed through cross-priming from other penaeids: *F. chinensis*, *L. vannamei* and *P. monodon*.

	Resource species	Locus	Primer sequence (5′– 3′)	Repeat motif	Size (bp)	T_a (°C)	*F. indicus* Repeat motif	*F. indicus* Size (bp)	*F. indicus* T_a (°C)	N_a	H_o	H_e	Accession number
1	*F. chinensis*	Fc07a	F: TCTTCGCCAGGAAACAG R: GCGGTCACACAAGCATA	$(TC)_{19}$. . .$(TC)_{33}$	138–168	53	$(TC...CT)_{34}$	242–309	53	6	0.431	0.723	JF715244
2		Fc22	F: TTGAACCTTCGTTAGTCC R: CGGGTGGAAATACAAATA	$(GT)_{50}$	202–246	50	$(GT)_{25}$... $(TCATAT)_3$	242–309	52	9	0.712	0.938	JF715246
3		Fc27	F: CGACCATTTTCGGTGTTC R: GCTGCGATAATTGAGACG	$(AG)_{37}$	–	51	$(AG)_{28}$	183–237	50	8	0.796	0.862	JF715248
4		Fc04	F: TGCTTTAATGGTTGCTG R: TACCAAGAATGGAGTG	$(TTA)_{18}$	199–238	55	$(TTA)_{21}$	309–404	50	12	0.825	0.918	JF715243
5		Fc24	F: ATATGGAAGTTCCTTTTG R: CTATGCTTATGTATCTGTCA	$(TG)_{40}$	191–247	55	$(TG)_{32}$	180–210	55	13	0.342	0.688	JF715247
6		Fc10	F: GGTCTTCGCCGACTCAGA R: CCCACCATCTCATCCACC	$(TG)_{10}$	211–235	56	$(TG)_{13}$	123–147	53	7	0.500	0.643	JF715245
7		Hd2545	F: TTACGGACCAGGAGACAATACAC R: AGAGACCCGCAGATTTCACC	$(AG)_{30}$	142–180	52	$(AG)_{22}...(GGA)_3$	309–404	50	8	0.624	0.792	JF715250
8		Hd2803	F: TTTCGTCGCTTCTTGACTTTG R: GCAGATTCACGAACGCAGTC	$(AT)_{27}$	245–273	50	$(ACAG)_6...(AT)_{23}$	238–242	52	5	0.653	0.854	JF297655
9		Hd3147	F: TGATTCGTAGTAGGTTCCAGCAT R: CATTTGCTTTGGGAGTGAGAGA	$(GT)_{36}$	234–286	52	$(GT)_{13}$	<350	55	7	0.725	0.846	JF715215
10		Hrd4353	F: AGACTGTGAGGCAAATCCCG R: AGGCACTTCATTTCGCTTACAG	$(AC)_{58}$	320–372	52	$(AC)_{40}$	309	52	6	0.605	0.640	JF715252
11		RS0676	F: ACGATGCTTATTAGCTGCG R: TGTGGAGCTTGATGGTTGC	–	–	55	$(AC)_{16}...(CA)_{20}$	160–300	55	3	0.816	0.935	JF715235
12		RS0622	F: TCAGTCCGTAGTTCATACTTGG R: CACATGCCTTTGTGTGAAAACG	–	–	55	$(TCTG)_{17}$	404–527	50	2	0.137	0.342	JF715236
13		IOPC04	F: TCTGGAAAGAAATGAAAGT R: AATACAACAATCCTTTAGTC	–	–	50	$(ATT)_4...(ATT)_{10}$	147–160	53	3	0.259	0.435	JF715237
14		Hrd3227	F: AGTGAAATGGATGGCTCTGG R: CAGAAAGGCATCAGTGGCAG	$(AT)_{42.5}$	235–286	50	$(AT)_{20}...(AT)_{15}$	180–190	50	12	0.643	0.746	JF715251
15	*L. vannamei*	HLJN-010	F: GCGGAGGAAGGAGGATAAAG R: TTGGTTTCTGAATTTGCGTATG	$(GA)_{37}(GAGACA)_6$	262–500	50	$(GA)_{28}...(GAGACA)_3$	350–400	50	8	0.474	0.661	JF715222
16		HLJN-014	F: GGGCGACAAATAAACGCATA R: TGTTATCTGCATTTTACGGCTTT	$(TG)_{10}A(GT)_5A$ $(TG)C(GT)_3(G)_3$	312–354	50	$(TG)_7...(TG)_3$	<450	50	2	0.197	0.351	JF715223
17		HLJN-023	F: AAGAGAGATGGAAGGAGTAAGTGC R: GATCAATACCTTGCAGCGAAA	$(AG)_3T(GA)_{24}A(AG)_{15}$ $AAA(GA)_2(AG)(GA)_9$	188–246	50	$(AG)_9...(AG)_{14}$	217–238	58	18	0.658	0.766	JF715224
18		HLJN-030	F: AAGTGTGTGAGCGAGTGTGG R: GTCGGGTCTTGGCTGTCA	$(GAGAGT)_5GTA$ $(TG)_{46}AC(AG)_{44}$	427–500	50	$(GT)_{31}...(GA)_{30}$	427–500	50	10	0.587	0.624	JF715225
19		Pvan0013	F: TGCTCTGGTAACGACAAACG R: AGACCTGTGGCGAAGTGC	$(TA)_5...(AT)_4$	282–284	50	$(TA)_7$	242–309	50	2	0.142	0.365	JF715227
20		Pvan0013	F: ATATTTCATGCGTTCCGAGG R: GACTATCTCACGCGCCTCTC	$(GT)_9(AT)_2(GT)_6$	145–149	56	$(TG)_{15}...(CTT)_3$	123–147	50	3	0.286	0.421	JF715233
21		Lvan055	F: TACTTGGACCTCAGTCA R: GCACGCTTAGTCTCAA	$(AAAAC)AAA$ $(AAAAC)_2$	199	50	$(TGA)_6...(TTTC)_5...$ $(ACAAA)_2$	309–404	50	21	0.889	0.904	JN185417

Table 1 (*contd*)

	Resource species	Locus	Primer sequence (5′– 3′)	Repeat motif	Size (bp)	T_a (°C)	*F. indicus* Repeat motif	Size (bp)	T_a (°C)	N_a	H_o	H_e	Accession number
22	*L. vannamei*	Lvan1	F: CCCTTTACCACCTCCTTCAATC R: AAGAGGAGGGGAAGGGTCAG	$(CT)_3$	166	50	$(CT)_8$	180–190	50	2	0.204	0.213	JF715218
23		Lvan2	F: CCATGGCTTTCCTCTTCTTTC R: AGGTAGGGAAGTCGTGAGGG	$(TCC)_5\ldots(CCT)_3\ldots$ $(CCT)_3\ldots(TC)_4\ldots(TC)_4$	327	50	$(TCC)_7$...$(CCT)_6$	309–404	50	4	0.397	0.521	JF715259
24		Lvan3	F: TGTCGTTAGTGCAGCTCATTC R: GGGGAGGAATAAGAGGAAAGG	$(TTC)_3TT(TTC)_3\ldots$ $(TCC)_3C(TCC)_5$	176	50	$(TTC)_{10}$	201	50	3	0.264	0.312	JF715260
25		Lvan4	F: GGCACACTGTTTAGTCCTCG R: CGAACAGAATGGCAGAGGAG	$(GTT)_3\ldots(GA)_3\ldots(TC)_3\ldots$ $(GT)_3\ldots(TC)_3\ldots(TC)_3$	242	50	$(TC)_8$	242	50	3	0.302	0.365	JF715261
26		Lvan5	F: AGACACATACAGACGCACGC R: GAGTTGCTCCCAAACGCTAC	$(AC)_3\ldots(AC)_3\ldots$ $(CA)_{19}\ldots(CA)_7\ldots$	329	50	$(CA)_{13}$...$(CA)_6$	309–404	50	3	0.342	0.403	JF715263
27		Lvan13	F: GAGAGCAAATAAGAAAGGGC R: AGGATGCAAATGATAACGAG	$(GGA)_3(GA)_3\ldots(CT)_3CC$ $(CT)_3CC(CCCT)_6(CT)_{10}$	219	50	$(GGA)_4$...$(TCCC)_3$... $(TC)_9$	180	50	11	0.432	0.683	JF715228
28		Lvan6	F: CACATCATGTCACTGCTACGAC R: GCTGCACAATCAACTTGCTTAC	$(AT)_3$	234	50	$(AT)_8$	309–404	50	2	0.197	0.351	JF715253
29		Lvan7	F: GAATGGGAGGAGAAGGATAG R: TTCCACGTGGTTTCCCGATG	$(AAC)_3(AG)_3\ldots(T)_{26}$	105	53	$(AG)_3..(T)_{18}$	123	53	2	0.168	0.224	JF715262
30		Lvan8	F: GAGAAGAGGCTGCTTTGTCG R: TGACTTTGAACTGGTGTGCG	$(TA)_3CAA(AT)_3$	278	50	$(TA)_7$	242–309	50	2	212	0.321	JF715234
31		Lvan9	F: GACGAACAGCCAGTCAACC R: GGGGATAGGGTAGCGGAAG	$(TC)_4$...$(TC)_4$...$(TC)_{14}$... $(TC)_3$	288	50	$(TC)_{14}$	242–309	50	4	0.365	0.412	JF297656
32		Lvan10	F: ATTCTTTGTGTTTCTTCGCC R: CGTCCCTGAAACTTTATCTCC	$(CA)_3\ldots(GT)_5\ldots$ $(GT)_3\ldots$	113	51	$(GT)_9$	147–160	50	2	0.186	0.261	JF715220
33		Lvan11	F: AGAGTCCTTGGTGAGTAGC R: GAGCGATAGAGTGCAATAAAG	$\ldots(TC)_{11}TTTTCTATA$ $(TC)_{11}$	353	53	$(TC)_{15}$	309–404	50	3	0.295	0.361	JF715221
34		Lvan12	F: ACACACCCATCCAACTACCC R: GGCCTATGGTTTGTCTGAGG	$(CA)_{35}AAC(ACGC)_4\ldots$ $(CA)_3A(ACCC)_3(AC)_3$	321	55	$(CA)_{17}..(ACCC)_3..$ $(CACG)_4$	<309	50	4	0.435	0.498	JF715219
35		Lvan0512	F: TGCCAGTGCCATTTGA R: CCTCCTCCTCCCAACT	$(TAT)_4TTT(TAT)_2$	258	50	$(TAT)_6$	309	50	7	0.447	0.899	JN185428
36		Pvan14	F: CTCCAGGACCGATAATGAGG R: CGACAGTCAAAACAAACATCC	$(TC)_3\ldots(TCG)_3\ldots$ $(TC)_{25}$	118	55	$(TCG)_3$...$(TC)_{23}$	404	55	4	0.562	0.597	JN185429
37		Pvan15	F: CTACTTATCGGTCTTTCTACTTACC R: CTTAGTGTTTTGTTCACCCC	$(TG)_4(CG)_3\ldots(AC)_7$ $(ACGC)_3GC(AC)_{28}\ldots$	206	55	$(CA)_{24}$	242–238	55	2	0.142	0.268	JN185430
38		Lvan01	F: TGTCTGAAGAGGGACTCGTG R: TTGTGCATTGTGGGTTTTTC	$(GT)_6\ldots(AC)_3(AT)_5\ldots$ $(AT)_{27}$	180	50	$(GT)_6$....$(AT)_{24}$	160–180	55	6	0.584	0.632	JF715238
39		Lvan051	F: GAGTTCCAATGTAAGTAG R: AAAATGTAGGTCGGTC	$(A)_7T(A)_3G(A)T(A)_4T(A)_4$	124	50	$(A)_7T(A)_3G(A)T$ $(A)_4T(A)_4$	123–147	55	2	0.139	0.311	JF715239
40		Lvan052	F: AGCCAGGAAGAGGAGG R: CATCGCCAGAAAGACAG	$(GAGC)_4$	112	50	$(T)_6\ldots.(T)_6$	110–123	55	2	0.211	0.325	JF715240
41		Lvan053	F: TTACGGGTGAAGTGTT R: TTTATGCTTCCCTACC	$(AC)_7$	289	55	$(AC)_8$	309–404	55	2	0.172	0.241	JF715241

Table 1 (*contd*)

	Resource species	Locus	Primer sequence (5′– 3′)	Repeat motif	Size (bp)	T_a (°C)	*F. indicus* Repeat motif	*F. indicus* Size (bp)	*F. indicus* T_a (°C)	N_a	H_o	H_e	Accession number
42	*L. vannamei*	Lvan054	F: GAAGTGAGCTTGGCATCCA R: GTAGAGCAGCGAGCCAGC	$(TC)_4CC(TC)_5$	109	50	$(ACG)_3\ldots(GGGC)_2C(GGGC)_2$	309–404	55	2	0.152	0.351	JF715242
43		Lvan056	F: CTTCATACCCATTCTTTCT R: GCAATAGGCTACAGTTCC	$(CTTC)_4$	300	50	$(AT)_9$...$(TGG)_8$...$(CCTC)_3$	<309–404	55	4	0.468	0.524	JF715212
44	(contd)	Lvan057	F: AAACCACCCTGACCATC R: CTGTGCCAAATTACAAGC	$(ATTTT)_4$	284	50	$(TTATTT)_3$...$(CAC)_3$	242–309	50	6	0.578	0.698	JF715213
45		Lvan058	F: TTGAAAAGCAAAGAAC R: CTTGGCAGGAGTAGTA	$(AT)_7$	200	55	$(AT)_6$	201–217	50	3	0.356	0.423	JF715264
46		Lvan059	F: GACTTGGAAGGGAACTG R: AGAAATAAAGGCTCTATGC	(AGAAAA)AA $(AGAAAA)_2AA$	100	50	$(AGAAAA)_2$	110	50	2	0.221	0.354	JF715214
47		Lvan0510	F: GCCATTTGATTGCTCT– R: TGACTTGGTCTTTGTTAG	$(GT)_8$	235	50	$(GT)_5$	238–242	50	5	0.353	0.528	JF715230
48		Lvan0511	F: GCAACTATTTATCATCTAAC R: TTCTGGAAGACTGTGG	$(AT)_9$	153	50	$(AT)_3$	160–180	50	3	0.358	0.438	JF715231
49		Lvan08	F: CTTCACAGAGGTTGGATAG R: CGATAAGGAAACTGACATTG	$(AGC)_8$	218–323	53	$(GCA)_{12}$	123–147	53	2	0.204	0.352	JN787962
50	*P. monodon*	PmMS7	F: CTCTCTCTCCGTCTTCCTG R: AGAGAGAGGAGCAGACTGG	$(CTGT)_4(CTAT)_{17}$... $(CTAT)_3(CAGT)_3(GTCA)_{24}$	310	55	$(TATC)_{13}$..... $(AGTC)_{23}$	160	50	2	0.196	0.309	JF715249
51		PmMS8	F: TTTGAGTCATAACTTCCAAGC R: TGCCATAAACTCTCTAACGAC	$(AAT)_9(AGT)(AAT)_5$	129	50	$(TAA)_{15}$	123–147	50	8	0.421	0.741	JF715265
52		PmMS11	F: GCAGCAACCAGGAAAGAGAG R: TGCAAGAAAGGGCAACTACA	$(TGAC)_4$. $(TGAC)_{24}$	201	50	$(TGAC)_{18}$	242–309	50	12	0.865	0.896	JF715266
53		PmMS16	F: TGGGCAGCGTGTGTGTAT R: AGACCGCTGCCGACTTAT	$(CATA)_{35}$	178	55	$(TATT)_3$...$(CAA)_2$	201–217	55	10	0.769	0.824	JN787956
54		PmMS18	F: CTGCTGGATTTAGAGAGTGG R: GATGATGTAAATGTAGATGCTGA	$(CAT)_{12}\ldots(CAT)_{25}(CAA)_{10}$	264	50	$CAT)_{23}$..$(CAA)_{10}$.. $(CAG)_{10}$	160–180	50	2	0.182	0.268	JF715254
55		PmMS1B	F: ACACGGAATCACCTCCCAGCCTAT R: TGTGTGTATGCGTGTGTGATTGTGG	$(TACA)_8$	120	50	$(TACA)_6$..$(ACAT)_3$	160–180	50	3	0.264	0.382	JF715255
56		PmMS2D2	F: TGCAGCTTCACACACCCATACACG R: TATGACAGGCAGTTGCGCCAGGTA	$(CAGT)_3(CGGT)_9(CAGT)_{25}$	310	50	$(GGTC)_7$...$(CAGT)_8$... (CAGT)7	217–238	50	14	0.885	0.914	JF715256
57		PmMS2G2	F: AGAGGTTTGCAGCCGAGCGAAAAG R: CGCTGATCCTGGCTTCTTGGAAAT	$(GACA)_{24}$	201	50	$(AGAC)_4$...$(AGAC)_7$	180	50	12	0.789	0.912	JF715257
58		PmMS9GG	F: ACAAGGAACCTGCCCAGAACATT R: CCCACCTCCTGCTTGGGAATATACA	$(CAA)_{22}$	245	50	$(CAA)_8CAG(CAA)_{10}$	180–190	50	11	0.579	0.783	JF715216
59		PmMS11AH	F: TGTCTGTGGCTCCCTGTCTCTCTG R: TGCCCTTGATACGAGCTTTATCTGTT	$(CACT)_{16}$	170	55	$(CACA)_8$	180–190	60	15	0.789	0.874	JF715217
60		PmMS7HG	F: GAAGGAGAAGGAGGAGGATTACAAGGA R: TCGGGCGGAGAATATGCAAATTAAA	$(GAT)_{54}$	318	60	$(GAT)_{41}$	309–404	60	18	0.684	0.933	JF715258
61		Pmon1	F: AAAACGGCGGTTGCTTCTC R: CCTCGGTATGGTGATGACATG	$(CT)_3$...$(CT)_6$	141–143	55	$(CT)_7$	147	55	4	0.468	0.628	JF715232
62		PM1713	F: GTTGCGACGGGTTGATTC R: TTTATGGCTATGGCTGACAC	$(CT)_{44}$	261–433	60	$(CT)_{36}$	147–160	55	17	0.605	0.861	JF715229

Table 1 (*contd*)

	Resource species	Locus	Primer sequence (5′– 3′)	Repeat motif	Size (bp)	T_a (°C)	*F. indicus* Repeat motif	*F. indicus* Size (bp)	*F. indicus* T_a (°C)	*F. indicus* N_a	*F. indicus* H_o	*F. indicus* H_e	*F. indicus* Accession number
63	*P. monodon*	PMC281	F: GGCAGGAATGTCAACCAAAT R: CCGGGTATAAACTACACATCAAAAC	T_{15}	171–175	55	$(T)_{11}$	160–180	55	5	0.621	0.751	JN185431
64		PMC311	F: CCATCAAAGTAAATCAGAACCAGAG R: TGAGTCTTGCAGCTCGAAAATA	$(AAT)_4$	115–118	55	$(TAAA)_6$	201–217	55	2	0.175	0.286	JN185432
65		PM138	F: ACGGAGTGGGTAGAGACATA R: ACAAGCGAAGTGAAGAGG	$(GT)_{47}$	268–338	56	$(GT)_{40}$	123–147	56	22	0.743	0.867	JN185418
66		PM205	F: AGGAATGATGGGAGGGAAAG R: AAGCTCAGGCAAGCGTGTAT	$(AG)_{23}$	153–209	56	$(CA)_{33}$	147–160	56	17	0.658	0.845	JN185419
67		PM580	F: AACTGCCTACAGTGTGTGCG R: GAATGGAGCCTGTTGGTTTG	$(AG)_{28}$	250–340	56	$(GA)_{29}$	147–160	56	23	0.889	0.934	JN185420
68		PM2345	F: GATATTTCAAGGAATGCTCG R: TAATTCGTGCCTTACCTCAT	$(TC)_{42}$	143–229	56	$(TC)_{32}$	160–180	56	20	0.675	0.839	JN185421
69		PM3538	F: GAACGTCGGGGGATTTACTT R: ACTATCACACCGAGGCTTGG	$(AC)_{12}$	371–441	56	$(CA)_{18}$	242–309	56	17	0.732	0.921	JN185422
70		PM3854	F: TCTTGGTCGGAATGGGTAAG R: TTCTGAGAAGGCACACATGC	$(GT)_{16} \ldots (GT)_{33}$	184–316	56	$(GT)_{37}$	123–180	56	18	0.668	0.845	JN185423
71		PM4018	F: GTTCCAAGCGACAGACGAGT R: CGAATGCACTGCCTGTATGT	$(AC)_{27}$	177–255	54	$(AC)_{24}$	217–242	54	20	0.787	0.922	JN185424
72		PM4089	F: CTTTTTGAAATCGCCCTGTT R: CATTCATCCCGCTCTTCTGT	$(CA)_{44}$	243–377	56	$(CA)_{39}$	242–309	56	25	0.825	0.906	JN185425
73		PM4798	F: GCTTGCGTGTGTGCATACTT R: GTTCCCCTCGTGTTTACGAA	$(TG)_{32} \ldots (TG)_{16}$	275–431	52	$(TG)_{27}....(TG)_{24}$	242–309	52	26	0.867	0.931	JN185426
74		PM4858	F: GCCTTGTTACGGTGGAGGTA R: CGGCCTATAACTGTCTGCCT	$(AC)_{16}$	215–295	55	$(CA)_{12}$	242–309	55	23	0.628	0.868	JN185427
75		PM4927	F: GGGGAATTAATCTGCCCATT R: AATGGCACAAGCAAAAGGAC	$(CA)_{25}$	296–362	53	$(AC)_{40}$	160–180	53	20	0.791	0.921	JN787960
76		PM5213	F: TGGACTGAGGTATGCAGCAC R: TCCTTGTTTGGAACCCTTTG	$(AT)_6 \ldots (CA)_{19}$	231–283	53	$(AT)_7(AC)_{18}$	242–309	53	15	0.687	0.782	JN787961
77		Pmo25	F: GGTGCGTGTTTGTCGTAAATACTGGC R: CATGCCCTTCCTTGACGCCAACCCTC	$(TG)_{21}$	132–206	53	$(TG)_{15}$	<242	50	26	0.845	0.896	JF715226
78		CU46	F: TGTGTAACAGCCTTCCCTGTGC R: TTTAGCCAACTACCTGGACAAGC	EST-SSR	295	55	$(AT)_6$	309–404	50	4	0.258	0.345	JN787957
79		CU73	F: TCTCAAGCATATCCACGGG R: AACACGTCATCACAAGCTGC	EST-SSR	226	55	$(TG)_6$	201–217	50	7	0.456	0.658	JN787958
80		CU135	F: CCTTCTTGGTGCTGTGACTG R: GCCTTCGTTTATCGCTTGTC	EST-SSR	178	55	$(CCCCG)_5$	160–180	50	6	0.524	0.628	JN787959
81		SAL96	F: GAAGGTGATGGTGGGTTCC R: TCTAAGCGGGGACTAACAGC	EST-SSR	143	55	$(GACT)_4$	160–180	50	2	0.146	0.421	JN977139

T_a, annealing temperature; N_a, number of alleles.

polyacrylamide gel (19 : 1 acrylamide : bisacrylamide) and visualized through silver staining. The alleles were designated according to PCR product size relative to a known molecular weight ladder (*pBR*322DNA/*Msp*I digest). To confirm the occurrence of repeats, all the cross-amplified polymorphic microsatellite loci were analysed by cloning in TOPO vector (Invitrogen, Carlsbad, USA) and sequencing in forward and reverse directions. The sequencing was done with the automated DNA sequencing ABI Genetic Analyzer 3730 platform (Applied Biosystems, Carlsbad, CA).

The data were analysed using software Genetix 4.02 (Belkhir *et al.* 1997) to obtain allele frequencies, mean number of alleles per locus, expected (H_e) and observed (H_o) heterozygosity values. Tests for conformity to Hardy–Weinberg expectations (HWE) were performed using Markov chain method with parameters dememorization = 1000, batches = 100 and iteration = 100 (GenePop 4.1.1, Rousset 2008). The data was also analysed for genotype linkage disequilibrium between pairs of loci in a population based on null hypothesis (genotypes at one locus is independent of genotypes at other loci), using the Genepop 4.1.1 programe (Rousset 2008). The genotypes of the loci deviating from HWE were tested according to Van Oosterhout *et al*. (2004, 2006) using MICRO-CHECKER for genotyping errors because of nonamplified alleles (null alleles).

Results and discussion

Screening of 396 microsatellite primers generated 102 successful amplifications (25.76%) for the target species in standard PCR conditions. After sequencing of 716 clones (102 loci in seven individuals each), 81 loci (20.45%) were confirmed as microsatellites with repeat sequences and 21 loci were nonrepeating EST markers. Among the 81 microsatellites, seven were EST-SSR markers and the remaining 74.24% were either failed or weakly amplified. The percentage of cross-amplification was 25.75%.

This is the first report of microsatellite marker development carried out in *F. indicus* through cross-species amplification from related species. Similar successful cross-species amplification for microsatellites in *Penaeids* were reported previously by Xu *et al.* (1999) and Freitas *et al.* (2007). The cross amplification success percentage of microsatellites in this study was low owing to the mutations in the sequences flanking microsatellite repeats. The reports of Moore *et al.* (1999) gave evidence for a low level of sequence similarity in microsatellite regions among penaeid shrimps. As per Bezault Etienne *et al.* (2012), the phylogenetic relationships and evolutionary distance between the different groups used for cross amplification from the target species reflect the success of cross-species amplifications. Likewise, in the present study, the success percentage was more with the closely related *Fenneropenaeus chinensis* (37.8%), then with *Penaeus monodon* (34.4%) and least with the distant species, *Litopenaeus vannamei* (14.3%). Similar results were observed in other fish and crustacean species (Gopalakrishnan *et al.* 2004, 2006; Jones *et al.* 2004; Chauhan *et al.* 2007; Chen *et al.* 2012; Huang *et al.* 2012; Guo *et al.* 2012; Kathirvelpandian *et al.* 2014; Mohitha *et al.* 2014).

Of the 81 developed microsatellite loci (table 2; 14 loci from *F. chinensis*, 35 loci from *L. vannamei* and 32 from *P. monodon*), 47 (58.02%) were perfect, 20 (24.69%) were compound and the remaining 14 (17.28%) were complex in nature. Based on Weber (1990), perfect SSRs were the predominant types of repeats than the compound and complex types. The present study agrees with this. Among the loci,

Table 2. Summary of microsatellite markers developed in *F. indicus*.

Total no. of markers screened for cross-priming		396
No. of loci cross-amplified in *F. indicus*		102
Percentage of cross-amplification		25.75%
No. of polymorphic microsatellite loci		81 (20.45%)
No. of alleles		2–26
Average no. of alleles		8.221
Molecular weight/allele size range		110–527 bp
Expected heterozygosity (H_e)		0.213–0.938
Type of repeat	Number	Percentage
Mononucleotide	03	3.70
Dinucleotide	51	62.96
Trinucleotide	13	16.05
Tetranucleotide	12	14.81
Pentanucleotide	02	2.47
Hexanucleotide	04	4.94
Type of repeat	Number	Percentage
Simple/perfect	47	58.02
Compound	20	24.69
Complex	14	17.28

dinucleotide repeats were dominant (62.96%), as in *P. mondon* (67%) (Xu *et al.* 1999) and *F. chinensis* (63.8%) (Wang *et al.* 2005; Dong *et al.* 2006; Gao *et al.* 2008). Exceptionally, trinucleotide repeats were found to be dominant in *Saccharum* spp. (Cordeiro *et al.* 2001).

Trinucleotide (16.05%), tetranucleotide repeats (14.81%), a few mononucleotide repeats (3.70%), pentanucleotide repeats (2.47%) and hexanucleotide repeats (4.94%) were also found (table 2). The relationship between polymorphism and the type of SSR motif is still not determined in penaeids (Wang *et al.* 2005). Weber (1990), in his study on the human genome, demonstrated high polymorphism of trinucleotide and tetranucleotide microsatellite with stable inheritance. In *F. indicus*, 16.05% trinucleotide and 14.81% tetranucleotide repeats were observed with high polymorphism and stable amplification. The highest level of polymorphism was observed in dinucleotide and trinucleotide perfect repeat motifs. Development of microsatellite markers with trinucleotide and tetranucleotide repeats are more valuable as errors while scoring due to the presence of stutter bands with dinucleotide repeats is more and can be avoided with trinucleotide and tetranucleotide repeats (Wang *et al.* 2005).

The repeat length varied from three (Lvan057, Lvan0511 and PmM16) to 41 (PmMS7HG) with the average length of 26.08. The tandem repeat sequence of 86.42% of the microsatellite loci were same as that of the resource species, while repeat motifs of 13.58% loci differed from that of the resource species which may be due to the faster repeat evolution without changing the flanking regions, as reported in fishes by Zardoya *et al.* (1996).

The level of polymorphism usually expressed as the number of alleles and the gene diversity (the expected heterozygosity). From the cross-species amplification trails, 2–26 alleles were observed in each locus with an average of 8.221 alleles per locus and the observed heterozygosities from 0.137 to 0.889. Following the sequential Bonferroni adjustment, the probability test did not detect any significant deviation in allele frequencies from that expected under ($P < 0.001$) HWE. None of the loci showed significant linkage disequilibrium for all pairs of loci ($P > 0.05$). It was therefore assumed that allelic variation at microsatellite loci could be considered independent. The estimated null allele frequency was not significant ($P < 0.05$) at all tested loci using different algorithms in MICRO-CHECKER, indicating the absence of null alleles. The expected heterozygosity of polymorphic loci ranged from 0.213 to 0.938, similar to the results observed among other shrimps from previous studies (Zhi-Ying *et al.* 2006; Gao *et al.* 2008) indicating the usefulness of these markers in population structure studies and mapping in *F. indicus*.

The polymorphic microsatellite DNA developed for *F. indicus* will provide a valuable resource for commercial shrimp breeding and selection programmes and genetic studies including stock identification, diversity assessments, linkage mapping and parentage analysis in both wild and cultured stocks of *F. indicus*.

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