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Characterization of genome-wide microsatellite markers in rabbitfishes, an important resource for artisanal fisheries in the Indo-West Pacific

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Abstract Rabbitfishes are reef-associated fishes that support local fisheries throughout the Indo-West Pacific region. Sound management of the resource requires the development of molecular tools for appropriate stock delimitation of the different species in the family. Microsatellite markers were developed for the cordonnier *Siganus sutor*, and their potential for cross-amplification was investigated in 12 congeneric species. A library of 792 repeat-containing sequences was built. Nineteen sets of newly developed primers, and 14 universal finfish microsatellites were tested in *S. sutor*. Amplification success of the 19 *Siganus*-specific markers ranged from 32 to 79% in the 12 other *Siganus* species, slightly decreasing when the genetic distance of the target species to *S. sutor* increased. Seventeen of these markers were polymorphic in *S. sutor* and were further assayed in *S. luridus*, *S. rivulatus*, and *S. spinus*, of which respectively 9, 10 and 8 were polymorphic. Statistical power analysis and an analysis of molecular variance showed that subtle genetic differentiation can be detected using these markers, highlighting their utility for the study of genetic diversity and population genetic structure in rabbitfishes.

Keywords Microsatellite resolution; Short-tandem repeat; Cross-species amplification; *Siganus* spp.

Introduction

Rabbitfishes (genus *Siganus*) are Indo-West Pacific reef-associated fishes currently comprising 30 valid species [1,2]. These fishes are economically important as a food source in coastal areas across the Indo-West Pacific where they support local fisheries and aquaculture [3,4,5,6,7]. Overfishing threatens this resource, as in the case of the cordonnier *Siganus sutor* (Valenciennes 1835), along the coasts of eastern Africa [8,9]. Appropriate resource management requires the identification of demographically independent populations, which is often achieved using microsatellite markers [10]. The aim of the present study was to develop microsatellite markers for siganid fishes. To this end, we identified polymorphic microsatellite loci in a target species (*S. sutor*) and evaluated their utility as genetic markers in twelve other congeneric species.

Materials and methods

Siganus spp. fin tissue samples were obtained from several locations across the Indo-West Pacific (Online Resource 1). The rabbitfish species included in this study were *S. argenteus*, *S. canaliculatus*, *S. corallinus*, *S. doliatus*, *S. fuscescens*, *S. laqueus*, *S. luridus*, *S. puellus*, *S. punctatus*, *S. rivulatus*, *S. spinu*s, *S. sutor* and *S. vulpinus* (Online Resource 1). Genomic DNA was extracted from fin tissue by using the DNeasy kit (Qiagen, Valencia CA, USA) following the manufacturer's protocol.

The microsatellite library was developed for *S. sutor* using the protocol of fast isolation by amplified-fragment length polymorphism of sequences containing repeats, which involves a protocol based on digestion and ligation reactions [11] combined to 454 sequencing [12]. We followed the same procedures as [13]. Genomic DNA was fragmented by enzyme digestion using the restriction enzyme *MseI* (New England Biolabs, Ipswich, MA). This was followed by the ligation of the small fragments to an adaptor (5'-TACTCAGGACTCAT-3'/5'-GACGATGAGGTCCTGAG-3') [11,13]. The ligation products were then hybridized to the repeat containing biotinylated probes ($CAAA_6$, $GAAA_6$, and $GATA_6$) [14] and the hybridized regions were captured on streptavidin-coated beads (Roche, Basel, Switzerland). After hybridization, non-specific binding elements were removed by washing. The eluted DNA, which consists of chemically and heat-separated DNA fragments containing selected repeats removed from the probes, was amplified according to [11] and the amplification product was pyrosequenced using GS-FLX titanium reagents by the 454 Genome Sequencer FLX™ (Inqaba Biotech, Pretoria, South Africa).

The repeat–enriched sequence library was mined for potential microsatellites, i.e., nucleotide sequences containing ≥ 8 short tandem repeats for all the repeat types including mono-, di-, tri-, tetra-, penta- and hexa-nucleotides, using MSATCOMMANDER v. 0.8.2 [15]. Sequences > 100-bp long were selected to ensure adequate flanking regions for primer design. Duplications were verified using the online version of the alignment program MAFFT (http://mafft.cbrc.jp/alignment/server/), selecting the default settings, but choosing the option for adjusting the direction of nucleotide sequences. A single copy of identical sequences was kept. The program PRIMER DESIGNER v. 4.20 (SECentral, Scientific & Educational Software, Durham NC, USA) was used to generate 59 primer sets based on standard criteria (Online Resources 2–4). A total of 19 of these primer sets were selected for further tests. Additionally, we included 14 microsatellite markers that universally amplify in acanthopterygian fishes [16]. All 33 markers were used for cross-species amplification in *S. sutor* and the 12 other siganid species. A total of 17 markers found to be polymorphic in *S. sutor* were further genotyped in three other species, *S. spinus*, *S. luridus* and *S. rivulatus*.

Amplification reactions were done in 10 µL volumes and the reaction mixture included ~ 40 ng of template DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Promega, Madison WI, USA), 0.2 pM primers (Whitehead Scientific, Johannesburg, South Africa), and 0.04 U Supertherm Taq polymerase (Southern Cross Biotechnologies, Cape Town, South Africa). The PCR cycling conditions were: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 1 min at annealing temperature (details in Online Resource 3), 72 °C for 1 min and by a final elongation at 72 °C for 7 min. Amplification products were subjected to electrophoresis on 3% agarose gels (1.5 h, 100 V) and visualised with GelRed™ stain (Biotium, Hayward CA, USA). The amplification reactions were repeated using fluorescently-labelled ChromaTide® Alexa Fluor™ 488-5-dUTPs (Invitrogen, Carlsbad CA, USA) to confirm the presence of multiple alleles at each locus. Forward primers of *Siganus*-specific loci that were polymorphic were labelled by the fluorescent G5 dye set (Applied Biosystems, Foster City CA, USA). Genotyping was done using the Quantitect Multiplex PCR kit (Qiagen), following the manufacturer's recommendations. The fragments were analysed on an ABI 3500xl genetic analyzer (Applied Biosystems) with the GeneScan Liz™ 500 Size Standard (Applied Biosystems). Scoring of genotypes was done using the GENEMARKER v. 1.5 software (SoftGenetics, State Collage, Pennsylvania, USA).

To investigate the transferability of the 19 new *Siganus*-specific markers across the family, we evaluated the relationship between genetic distance to the target species *S. sutor* and cross-species amplification success. To estimate genetic distance, nucleotide sequences of the mitochondrial *cytochrome b* (*cytb*) gene were used. This included sequences available from GenBank (Online Resource 5) and new sequences generated in the present study (Online Resource 6). We followed the procedure described in [17] to design specific primers for the family Siganidae. Cycling conditions included initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 1 min; and final elongation at 72°C for 7 min. The amplified products were precipitated using 2.8 volumes of absolute ethanol, 0.4 volumes of Sabax water and 0.08 volumes of 3M sodium acetate, followed by a washing step using 90 µL 70% ethanol. The products were eluted in 15 µL Sabax water. Cycle sequencing reactions contained approximately 30 ng purified DNA, 1 µL ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems), 5 x sequencing buffer and 3.2 pM primer. Sequencing was done on an ABI 3500xl sequencer (Applied Biosystems).

Genetic distance between species was estimated as the net mean nucleotide divergence (Kimura-2-parameter model; MEGA6) [18] at the *cytb* locus between *S. sutor* and the other species considered. The distances were used to reconstruct a Neighbor-Joining tree in MEGA6, to illustrate the diversity of species used in the present study and their phylogenetic relationships (Fig. 1a). The GenBank sequences that represented possibly misidentified specimens, possible mitochondrial-DNA introgression or cryptic species were removed from the analysis (Online Resource 5).

Summary statistics on the microsatellite genotype data, and their significance, were estimated using GENETIX v.4.05 [19]. The possible presence of null alleles was assessed using MICROCHECKER v. 2.2.3 [20]. Linkage disequilibrium (LD) among markers was estimated and tested using permutation tests in ARLEQUIN v. 3.0 [21]. BAYESCAN v. 2.1 [22] and LOSITAN [23] were used to detect potential outlier loci based on allelic differences estimated by F_{ST}, and comparison between the observed F_{ST} and expected heterozygosity among the populations, respectively. We used the program POWSIM v. 4.1 [24] to evaluate the statistical power of the markers to detect subtle genetic structure. This program identifies the threshold above which F_{ST} values become significant, given the distribution of allele frequencies in a set of markers. The specific test that we performed relied on simulated pairs of populations (100 replicates) with a set sample size (50 individuals) and

effective population size (N_e = 5000), but a varying number of generations since isolation (t = 5–300) to generate various levels of divergence (i.e. F_{ST} values). To further evaluate the utility of the markers with regards to potential spatial genetic structuring, an analysis of molecular variance (AMOVA) [25] was done among samples of *S. spinus* and *S. luridus*, the two species from the study with multiple samples from different locations. Each sampling location was defined as a population (see Online Resource 1). The significance of the variance components among populations and within populations was determined using 1000 permutations.

Results and discussion

The repeat-enriched library contained 6112 sequences and the mining step uncovered 310, 61, and 421 sequences containing di-, tri- and tetra-nucleotide repeats, respectively (Online Resource 7). Only a subset of these sequences was longer than 100 bp and contained sufficiently long flanking regions at both ends. These included 28, 12, and 38 sequences containing di-, tri-, and tetra-nucleotide repeats, respectively. Among these, 59 sequences were used for primer design (Online Resource 2). Due to budget limitations, only 19 of these potential markers were tested for amplification in 13 *Siganus* species, including *S. sutor*.

Cross-species amplification success of the 33 markers (of which 19 were *Siganus*-specific and14 were universal in ray-finned fishes) across 12 siganids ranged from 36% (*S. laqueus*) to 76% (*S. corallinus*, *S. doliatus* and *S. fuscescens*). The genetic distance to *S. sutor*, estimated from nucleotide sequences at the *cytb* locus, ranged from 0.035 (*S. rivulatus*) to 0.165 (*S. argenteus*) (Online Resource 8).The amplification success of the 19 *Siganus*-specific markers ranged from 32% (in *S. vulpinus*) to 79% (in *S. fuscescens*) (Fig. 1a) and showed a weak yet significant decrease with the genetic distance to *S. sutor* (Fig. 1b). The new microsatellite markers therefore amplify in species other than *S. sutor* but phylogenetic distance affects the amplification efficiency. Alternative primers might need to be designed for the few markers that do not, or poorly amplify in some species. Similar negative correlation has been reported previously [13,26]. This trend is likely a general one across taxa and markers, and should be accounted for in the evaluation of cross-species amplification.

Twelve of the 19 *Siganus*-specific markers and five of the universal acanthopterygian fish markers were polymorphic in *S. sutor* (Online Resource 3). These were tested for polymorphism in three other siganid species (Table 1). The expected heterozygosity averaged across all loci was 0.822 for *S. luridus*, 0.753 for *S. rivulatus*, 0.682 for *S. spinus*, and 0.823 for *S. sutor*, while the mean number of alleles across loci was 8.78, 5.73, 6.75, and 11.69, respectively. Significant deviation from Hardy–Weinberg equilibrium was detected at 3/9 loci in *S. luridus*, 1/11 loci in *S. rivulatus*, 2/8 loci in *S. spinus*, and 7/17 loci in *S. sutor*, but null alleles were detected in only a smaller proportion of the loci: *SIG006* in *S. spinus*, and *SIG008* and *SIG013* in *S. sutor*. Significant linkage disequilibrium values were detected for 2/28 locus-pairwise comparisons in *S. spinus* (Online Resource 9) and for 5/120 comparisons in *S. sutor*, after Bonferroni correction [27] (Online Resource 10). Considering the low occurrence of significant values, both null alleles and linkage disequilibrium are expected to have a marginal effect on the analysis of genetic differentiation between populations in *S. spinus* and *S. sutor*. BAYESCAN [22] and LOSITAN [23] detected a single outlier locus (*SIG017*), relative to the level of genetic differentiation between populations. The occurrence at this locus of an F_{ST} value above the 99% confidence interval suggests directional selection, but this can also be due to non-overlapping allele sizes between species.

By assessing the resolution power of the microsatellite markers, we were able to evaluate whether the sets of microsatellites in *S. rivulatus*, *S. spinus* and *S. sutor* could potentially identify genetically distinct populations. A previous simulation study showed that the threshold F_{ST} values for the detection of genetic subdivision

among populations ranged from as low as 0·005–0·007 when using the appropriate set of markers [29]. In the present study, the set of microsatellite markers was able to detect subtle levels of genetic differentiation through the power analysis. These values were as low as F_{ST} = 0.0135 in *S. luridus*, F_{ST} = 0.015 in S. spinus, and F_{ST} = 0.011 in *S. sutor* (Fig. 2); *S. rivulatus* was excluded from this analysis due to low sample sizes. These results based on simulations were confirmed by the AMOVA analyses, which showed significant genetic differentiation among locations in both *S. luridus* (F_{ST} = 0.152, P = 0.001) and *S. spinus* (F_{ST} = 0.077, P = 0.021) despite low sample sizes per location ranging from one to ten (Table 2). The set of markers proposed in this study therefore proved useful in detecting subtle genetic structure in *Siganus* spp.

The amplification success, the level of polymorphism, the resolution power, and the pilot study for the detection of population differentiation show that these microsatellites represent a valuable molecular resource for rabbitfish species. These markers will be useful for developing studies aimed at improving resource management in artisanal rabbitfish fisheries across the Indo-West Pacific.

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Conflict of interest

We have no conflict of interest regarding the content of the present paper.

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Table 1 Summary statistics for 17 polymorphic loci tested in four siganids (with sample size in brackets) including the number of observed alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) estimates [28]

Locus	Statistic	Species			
		S. luridus (17)	*S. rivulatus* (7)	*S. spinus* (20)	*S. sutor* (32)
ORLA2-91					
	N_A	13	6	12	14
	H_E	0.927	0.929	0.840	0.905
	H_O	0.882	1	0.700	0.931
	F_{IS}	0.050	-0.091	0.191***	-0.029
ORLA8-113					
	N_A	8	1	11	4
	H_E	0.831	0	0.853	0.696
	H_O	0.625	0	0.900	0.594
	F_{IS}	0.254*	0	-0.030	0.149
ORLA11-71					
	N_A	5	2	4	5
	H_E	0.546	0.533	0.650	0.668
	H_O	0.588	0.400	0.700	0.719
	F_{IS}	-0.081	0.273	-0.051	-0.078
ORLA12-160					
	N_A	NA	MD	11	11
	H_E			0.837	0.883
	H_O			0.824	0.906
	F_{IS}			0.047	-0.027
ORLA14-42					
	N_A	15	5	5	17
	H_E	0.944	0.800	0.555	0.913
	H_O	0.857	1	0.650	0.844
	F_{IS}	0.096	-0.290	-0.146	0.077
SIG006					
	N_A	NA	7	3	8
	H_E		0.890	0.539	0.732
	H_O		1	0.300	0.406
	F_{IS}		-0.135	0.464***	0.449***
SIG007					
	N_A	7	4	NA	10
	H_E	0.836	0.714		0.780
	H_O	0.546	0.857		0.677
	F_{IS}	0.358***	-0.220		0.133
SIG008					
	N_A	NA	NA	NA	11
	H_E				0.882
	H_O				0.308
	F_{IS}				0.655***
SIG009					
	N_A	MD	11	MD	23
	H_E		0.956		0.953
	H_O		1		0.739
	F_{IS}		-0.050		0.228***
SIG010					
	N_a	MD	MD	NA	16
	H_E				0.886
	H_O				0.636
	F_{IS}				0.286***
SIG011					
	N_A	9	7	5	16
	H_E	0.874	0.933	0.709	0.937
	H_O	0.769	1	0.600	0.760
	F_{IS}	0.124	-0.081	0.178	0.192***
SIG013					

	N_A	MD	NA	MD	5
	H_E				0.658
	H_O				0.044
	F_{IS}				0.935***
SIG014					
	N_A	12	8	MD	MD
	H_E	0.909	0.912		
	H_O	0.688	0.714		
	F_{IS}	0.250*	0.231		
SIG015					
	N_A	NA	NA	NA	15
	H_E				0.899
	H_O				0.781
	F_{IS}				0.133*
SIG017					
	N_a	5	6	3	6
	H_E	0.708	0.747	0.471	0.691
	H_O	0.647	0.714	0.400	0.625
	F_{IS}	0.088	0.048	0.176	0.096
SIG019					
	N_A	NA	NA	MD	12
	H_E				0.806
	H_O				0.781
	F_{IS}				0.031
SIG020					
	N_A	5	6	NA	14
	H_E	0.822	0.864		0.880
	H_O	0.800	0.500		0.857
	F_{IS}	0.030	0.444*		0.044

* $P < 0.05$; *** $P < 0.001$. *NA*: locus with no amplification, *MD*: locus with missing data (a proportion of the individuals were not amplified)

Table 2 Analysis of molecular variance for *Siganus luridus* and *Siganus spinus*. Degrees of freedom (df), percentage contribution to overall genetic variance (GV%) and fixation index (F_{ST}) with related *P*-value are presented.

Species	Source of variation	df	GV%	Fixation index	*P*-value
S. luridus	Among populations	2	15.19	F_{ST} : 0.152	0.001
	Within populations	31	84.81		
S. spinus	Among populations	4	7.67	F_{ST} : 0.077	0.021
	Within populations	35	92.33		

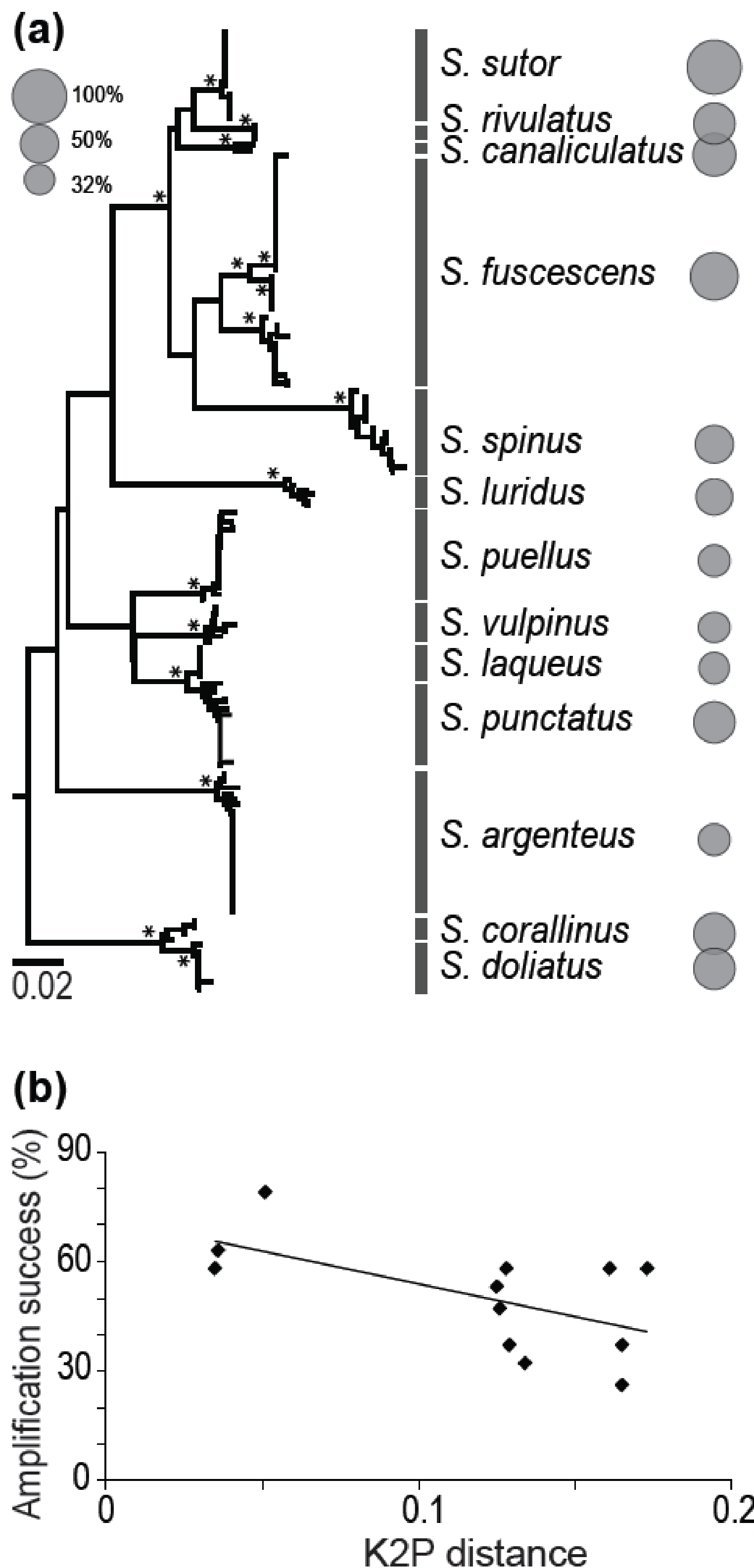

Fig. 1 Amplification success of the new set of 19 microsatellites in 13 *Siganus* species. **a** Genetic relationships among 13 *Siganus* species illustrated by a Neighbour Joining tree based on 184 *cytochrome b* gene sequences [MEGA6: Kimura-2 parameter (K2P) model; pairwise deletion] [18]. The amplification success of the 19 loci represented by circles whose surface is proportional to the proportion of amplifying loci. Supported nodes (≥ 75% bootstrap score) are indicated with a star. *cytb* gene sequences of three surgeonfish species (*Acanthurus blochii*, *A. triostegus*, *Naso unicornis*) were chosen as outgroup. **b** Amplification success plotted against K2P distance to *S. sutor*. Linear regression model: $y = -0.0178x + 0.7172$, $R = 0.3531$. The null hypothesis that the slope is equal to 0 was rejected ($F = 5.46$; $P = 0.042$).

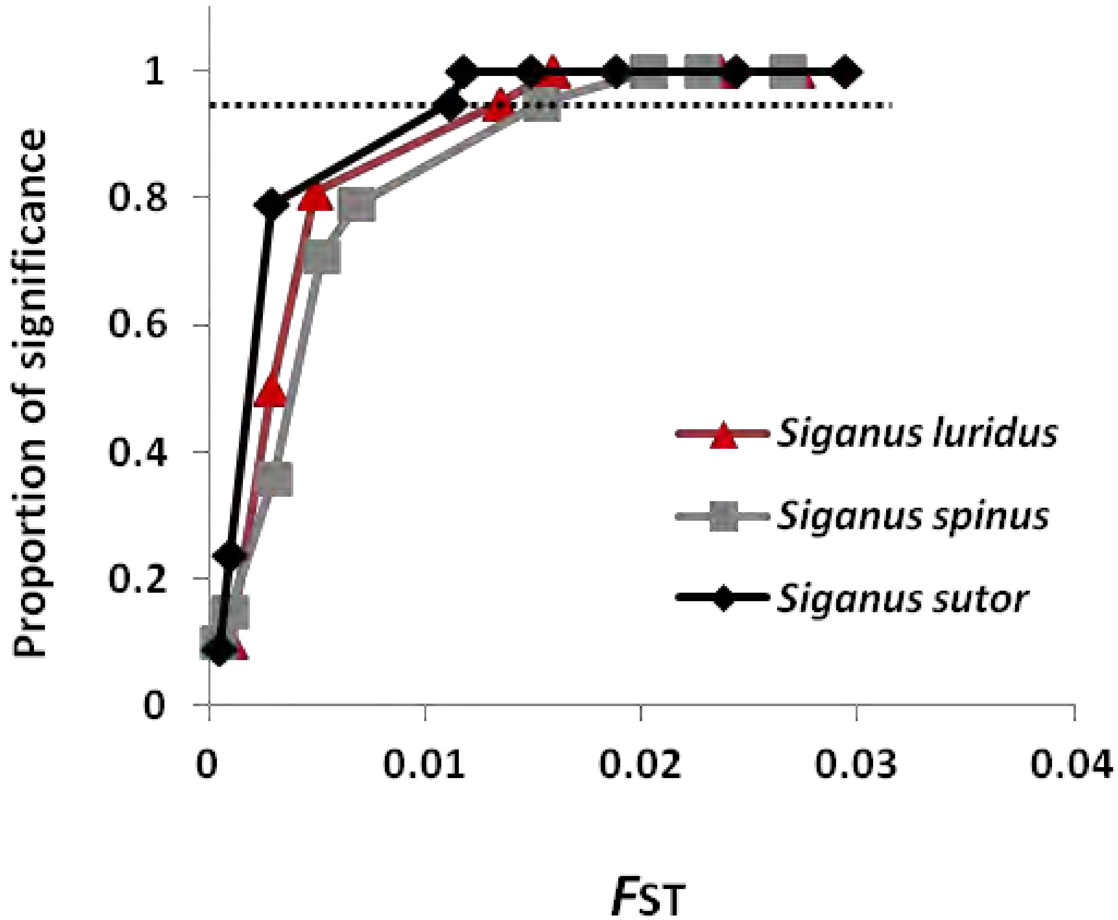

Fig. 2 Power analysis for the set of polymorphic microsatellite markers illustrating the proportion of significance (*PS*) as a function of the F_{ST} value in *S. luridus* (9 loci; 17 individuals; $PS = 2.076 + 0.261. \ln(F_{ST})$; $F_{ST95} = 0.0135$), *S. spinus* (8 loci; 20 individuals; $PS = 2.055 + 0.265. \ln(F_{ST})$; $F_{ST95} = 0.015$), and *S. sutor* (16 loci; 32 individuals; $PS = 1.991 + 0.232. \ln(F_{ST})$; $F_{ST95} = 0.011$). Dotted line indicates 95% significance threshold (F_{ST95}). The relatively low F_{ST} values observed at this threshold show that fine-scale population structure can be detected using this set of markers

Molecular Biology Reports

Supplementary information to:

Characterization of genome wide microsatellite markers for rabbitfishes, an important resource for artisanal fisheries in the Indo-Pacific

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Online Resources 1-10 here appended

Online Resource 1
List of rabbitfish, *Siganus* spp. specimens sub-sampled for genetics, with sampling details. *MNHN*: Muséum national d'histoire naturelle, Paris.

Species	Specimen no.	Sampling date	Sampling locality	Collector
S. argenteus				
	IFA0001	11 Dec. 2013	Mangily, Ifaty, Madagascar	S. Abeare
	S006	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
S. canaliculatus				
	ScanJurong02	21 Nov. 2006	Jurong fish market, Singapore (from off Phuket, Thailand)	P. Borsa
	ScanJurong03	21 Nov. 2006	Jurong fish market, Singapore (from off Phuket, Thailand)	P. Borsa
S. corallinus				
	T-392	30 Apr. 2005	SE side, Conception Is., Seychelles	G. Gouws
	S002 (MNHN 2007-0006)	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
S. doliatus				
	S003 (MNHN 2007-0007)	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
	S007 (MNHN 2007-0008)	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
S. fuscescens				
	Sfus04	June 2006	Marau, Solomon Islands,	E. Tardy
	Sfus05	June 2006	Marau, Solomon Islands,	E. Tardy
S. laqueus				
	T-66	16 Apr. 2005	Victoria market, Seychelles	G. Gouws
	RB09-241	01 Dec. 2009	Mangrove channel, Nampula, Mozambique	G. Gouws
	KEN0012	14 Mar. 2014	Msambweni, Kenya	N. Wambiji
	KEN0013	14 Mar. 2014	Msambweni, Kenya	N. Wambiji
S. luridus				
	KEN0002	01 Apr. 2014	Kilifi, Kenya	N. Wambiji
	KEN0003	01 Apr. 2014	Kilifi, Kenya	N. Wambiji
	KEN0010	13 Mar. 2014	Msambweni, Kenya	N. Wambiji
	KEN0011	13 Mar. 2014	Msambweni, Kenya	N. Wambiji
	KEN0018	11 Feb. 2013	Mombasa, Kenya	N. Wambiji
	KEN0019	11 Feb. 2013	Mombasa, Kenya	N. Wambiji
	KEN0022	17 Jan. 2014	Malindi, Kenya	N. Wambiji
	KEN0023	17 Jan. 2014	Malindi, Kenya	N. Wambiji
	Slur01	2002	Lattaqieh, Syria	M. Hassan
	Slur02	2002	Lattaqieh, Syria	M. Hassan
	Slur03	2002	Lattaqieh, Syria	M. Hassan
	Slur04	2002	Lattaqieh, Syria	M. Hassan
	Slur05	2002	Lattaqieh, Syria	M. Hassan
	Slur06	2002	Lattaqieh, Syria	M. Hassan
	Slur07	2002	Lattaqieh, Syria	M. Hassan
	Ss01	-	La Réunion	E. Teissier
	Ss02	-	La Réunion	E. Teissier
S. puellus				
	S004	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
S. punctatus				
	S001 (MNHN 2007-0010)	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet
S. rivulatus				
	Sriv01	2002	Lattaqieh, Syria	M. Hassan
	Sriv02	2002	Lattaqieh, Syria	M. Hassan
	Sriv03	2002	Lattaqieh, Syria	M. Hassan
	Sriv04	2002	Lattaqieh, Syria	M. Hassan
	Sriv05	2002	Lattaqieh, Syria	M. Hassan
	Sriv06	2002	Lattaqieh, Syria	M. Hassan
	Sriv07	2002	Lattaqieh, Syria	M. Hassan
S. spinus				
	JNC 1555	28 June 2005	Récif Goéland, New Caledonia	G. Mou-Tham
	JNC 1556	28 June 2005	Récif Goéland, New Caledonia	G. Mou-Tham
	Sspi01	28 June 2005	Récif Goéland, New Caledonia	G. Mou-Tham
	Sspi02	13 June 2006	Ngela, Solomon Is.	E. Tardy
	Sspi03	19 June 2006	Ngela, Solomon Is.	E. Tardy
	Sspi04	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi05	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi06	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi07	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi08	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi10	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi11	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin

	Sspi12	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi13	27 Aug. 2006	Manus I., Papua New Guinea	P. Boblin
	Sspi14	21 Feb. 2007	Bali barat, Indonesia	F. Giancarlo
	Sspi34-1 2	27 Mar. 2006	Opunohu Bay, Moorea	D. Lecchini
	Sspi34-2 1	27 Mar. 2006	Opunohu Bay, Moorea	D. Lecchini
	Sspi34-2 2	27 Mar. 2006	Opunohu Bay, Moorea	D. Lecchini
	Sspi34-2 3	27 Mar. 2006	Opunohu Bay, Moorea	D. Lecchini
S. sutor				
	INS0001	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0002	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0004	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0005	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0006	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0007	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0008	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0009	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0011	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0012	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0013	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0014	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0015	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0018	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0019	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0020	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0022	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0023	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0024	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0025	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0026	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0027	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0028	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0029	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0031	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0032	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0033	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0034	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0035	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0036	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0037	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
	INS0038	31 Mar. 2014	Inhassoro, Mozambique	A. Brito
S. vulpinus				
	S005	04 Feb. 2005	Nouméa fish market, New Caledonia	A. Collet

Online Resource 2

Procedure of selection of the di-, tri- and tetra-nucleotide microsatellite markers from the sequences containing repeats to the design of primers

Statistic	Length of repeat		
	Di-nucleotide	Tri-nucleotide	Tetra-nucleotide
Number of sequences containing repeats	310	61	421
Number of sequences with adequate flanking regions	56	21	190
Number of sequences > 100bp in length with no duplicates	28	12	38
Number of sequences with designed primers	21	5	33

[a] Primers were designed using the program PRIMER DESIGNER v4.20 based on the standard criteria (melting temperature, GC content, presence of dimers, hairpin and false priming, differential stability between the 5' and 3' ends, and presence of adjacent repeats of the same base).

Online Resource 3

Details of the 17 polymorphic markers genotyped in *Siganus sutor*, including locus name, fluorescent dye, repeat motif, annealing temperature (T_A) and the allele size range in base pairs

Locus	Dye	Repeat motif	T_A (°C)	Allele range (bp)
ORLA2-91 [b, c]	NED	$(GT)_7$	60	134-174
ORLA8-113 [b, c]	PET	$(CA)_{13}$	60	161-167
ORLA11-71 [a, c]	6-FAM	$(CA)_8$	60	377-387
ORLA12-160 [b, c]	PET	$(CA)_{12}(TA)(CA)_6$	60	283-303
ORLA14-42 [b, c]	PET	$(CA)_{10}$	60	368-400
SIG006 [a]	NED	$(CAAA)_9$	55	215-251
SIG007 [a]	VIC	$(CAAA)_5(CAGA)(CAAA)_8$	55	191-259
SIG008 [a]	6-FAM	$(ATAG)_4(GTAG)(ATAG)_{11}$	55	130-186
SIG009 [a]	PET	$(AGAT)_{18}$	55	196-352
SIG010 [a]	6-FAM	$(AGAT)_{12}$	55	238-282
SIG011 [b]	VIC	$(AGAT)_{16}$	55	184-268
SIG013 [b]	6-FAM	$(GTTT)_{11}$	57	266-282
SIG014 [a]	VIC	$(GTTT)_{11}$	55	333-369
SIG015 [b]	NED	$(CTTT)_{11}(CTCT)(CTTT)_6$	55	243-299
SIG017 [a]	PET	$(CAG)_8$	55	102-117
SIG019 [b]	6-FAM	$(GTT)_{12}$	55	187-235
SIG020 [a]	NED	$(ACC)_{15}$	57	115-157

[a] Multiplex combination 1
[b] Multiplex combination 2
[c] Primer set from Gotoh et al. (2013)

Online Resource 4

Details of the 59 microsatellite markers designed from the repeat-enriched library obtained for *Siganus sutor*, including locus name, forward and reverse primer sequences, repeat motif and size of microsatellite amplicon in base pairs

Locus	Forward primer 5' – 3'	Reverse primer 5' – 3'	Repeat motif	Amplicon size (bp)
SIG001	GTGGCACGAACAACACATGA	GGGCTTTGCAAGTCACGAAGTC	(ATCT)$_{10}$	231
SIG002 [a]	CTTGGCAAGCAATGGAAGC	AGTGAGTTGCCTGGAAAG	(ATCT)$_{21}$	308
SIG003 [a]	ACATTTAGCGTGGGAGAC	TTCTGCATCAGACTGAAC	(ATCT)$_{12}$	153
SIG004 [a]	CCTCCGTGGAAGTCAACAG	AATCATGGCGACTGTTGAGC	(GT)$_{9}$	175
SIG005 [a]	GTCGAAGGTCGTAGTTCTTG	GCATGCCTGTGACTGTCAAC	(ATCT)^{13}	261
SIG006 [a, b]	AACTGTGTGAGCGATCTG	TCAGTACAGCGTGACATC	(CAAA)$_{9}$	237
SIG007 [a, b]	AAACAAACGTCCGGAAAC	GGTCAGTCAGCATCGTAA	(CAAA)$_{5}$(CAGA)(CAAA)$_{8}$	265
SIG008 [a, b]	TGCTAAATGTCCCTTCAC	TGGATCTGTGCTACCTAC	(ATAG)$_{4}$(GTAG)(ATAG)$_{11}$	160
SIG009 [a, b]	TCCTGCTACCCACTCCTTC	ATTACAGGAAGCGGAAAC	(AGAT)$_{18}$	243
SIG010 [a, b]	ATCTGACGGATACGATTC	TCGAGCAGAATGGCACTCAG	(AGAT)$_{12}$	269
SIG011 [a, b]	TTCTCCTAGGCCTGGAGATG	CCTCCTCTAATGCACCTAT	(AGAT)$_{16}$	211
SIG012 [a]	GGCTCGTTGACCTCTCAG	AGAACAAACTCCGGAAAC	(GTTT)^{8}	293
SIG013 [a, b]	CGGCTCTTTGTGTCTGT	ACAATGTTGCGCTCACTC	(GTTT)$_{11}$	269
SIG014 [a, b]	TGGCTGTTCCTTACTGTCT	TATGACGGCCACTAGATG	(GTTT)$_{11}$	354
SIG015 [a, b]	GGGGAGAGAAATGATAGC	CCGCATTAGTAATTGCTC	(CTTT)$_{11}$(CTCT)(CTTT)$_{6}$	279
SIG016 [a]	GGCTATGCACTGATTCAC	ACATAATCCCATCAGCAG	(AGC)$_{8}$	124
SIG017 [a, b]	GAGTGGGTGTCCTGAATG	AGCATCAATAGACGTGTG	(CAG)$_{8}$	111
SIG018 [a]	ACTGATGAGCTGGTTTTC	TGCTGCTGTCAGCAGTTC	(AGG)$_{9}$	115
SIG019 [a, b]	AAGTAGCGCCACCTTCTG	TGACAGCTTGCAGCAGAA	(GTT)$_{12}$	198
SIG020 [a, b]	TCTGAACCATGAGCTTCC	TCGAACGGAGACGCTTCA	(ACC)$_{15}$	138
SIG021	CTTCTCGCCTTCCAGCTCATC	TGTGATGAGTACCTGAGTAA	(CTTT)$_{10}$	349
SIG022	GAGGCAGTGACAGCCTGTCT	GTAAGGTGCTGCAGCAGGATG	(CTTT)$_{14}$	189
SIG023	ACACAGGACCTGCAAGAGA	TGCGGATAGGCTCCAATGACC	(ATCT)$_{14}$	268
SIG024	ATGAGACCGTACATCTTCATC	TTGTTGGGTTTAGTTCTAGGA	(GT)$_{13}$	173
SIG025	CCAATGCAGCACATTCAGT	GGATGTTGTAGCAGCCTCCA	(GT)$_{11}$	130
SIG026	TTTCCTCTACCACCTCTCATC	CGAATGACTTCGCTGGAAC	(ATCT)$_{10}$	223
SIG027	TCAGAACCGCCTCTCACTGC	AAGAAGTACGAGTGATAGAG	(CT)$_{9}$	163
SIG028	GGGGGCGCAATAGTAAGATG	TCAGATTGGAGGCCACAATCC	(ATCT)$_{19}$	245
SIG029	TCCTCTGCCATTAGGTTCTG	GGCGTAAACTTACAGTACA	(GT)$_{9}$	150
SIG030	TTATGCCACACTGGAGGTTC	CCTCCCTTGATGTGGAGTAG	(GT)$_{11}$	160
SIG031	CCACAACACACATATACAC	GTGCAGCAGTAAGGTGGTCT	(ATCT)$_{15}$	204
SIG032	F:GAGGGCTTCTCTTACAGTAG	ATGGAGGCGAGAGGCAGAGA	(ATCT)$_{19}$	294
SIG033	CATCAACAGGTTGGCTACAG	GTACCAGCCTGGAAACTAGC	(GT)$_{8}$	156
SIG034	TCCTCATCGCACTCGCCAGA	ACGCAGATCGTGATGAGTC	(ATCT)$_{13}$	300
SIG035	GGCAGTGCTGGTGGATCTAC	ACGCAGAGGAAACACAGAAC	(GT)$_{9}$	152
SIG036	GTGGCCGTTCTTATATTATG	CTAATGAGGTCGAGTCC	(GT)$_{8}$	136
SIG037	CAGCGCTGATGGCACAATCC	CTTCTCTCCTTTTTTCTTAC	(GT)$_{18}$	224
SIG038	GGATGAAAACATCGGAGTC	GACTGCACAACACAGCTATGG	(AGAT)$_{15}$	193

SIG039	GTGGGGCCAATATCTGTGGG	AGCCTGGCCAATATCTGAGTG	(ATCT)$_{12}$	208
SIG040	TGACGACTAGGCTAGCAGAG	ACCCTCAGGCTCCAATACCTT	(AAAC)$_{13}$	194
SIG041	CTGGATGCACAGGTGTGTA	GGTTCACAGGTGGCTGCTGCTG	(AAAC)$_{13}$	236
SIG042	CTGGGTCGAGTCGCCCCC	TGCTTCTGTCGTTGCATCAG	(AAAC)$_{10}$	166
SIG043	ACCTGCCATCTGTATCATTC	CATGGCGGGAAACGTTCTGAGC	(AC)$_{8}$	122
SIG044	CGTAGCCCGTGGCCGTGTAG	CGTTAGCCCGTCCACGGCGT	(AC)$_{11}$	164
SIG045	AGTGTATGAGGACGAGCGG	TGTTGTCTCACTTTACAACAACAC	(AAAC)$_{9}$	236
SIG046	TCAACAACTGGAATCAACAAACTG	CTGATCTCCGTGCTCCACC	(AAAC)$_{13}$	248
SIG047	AGAAACAAACGTCCGGAAAC	CCTGAGTAATCATCTCCGGC	(AAAC)$_{8}$	311
SIG048	TGACTCATGCACAAGTACATCC	TTGTGGTAATATACAGGGCGG	(AAAC)$_{13}$	187
SIG049	ACACACTCCCCTATTCCTGC	TAACGTCTGGGACGAGAGC	(AGAT)$_{17}$	197
SIG050	TCACGCTCAGCAAGTAACC	ACTGGTGAGACTATTCTTGGC	(AAAC)$_{10}$	176
SIG051	ACTGTTGCCATAGTGAAGTGC	AGCGCACTGTGTCCATTAG	(AC)$_{8}$	161
SIG052	ACATTTATGTCTTAGCAACGGGG	ACAAAGTGGAGGCGAACTG	(AC)$_{11}$	181
SIG053	TCCCCACAGTGTTTCCACC	ACAGCTTTTGGCCCTTCAG	(AC)$_{10}$	232
SIG054	ACACTCTCACACAGACCTGC	ACGGCGAGCCTACTTACTTC	(AC)$_{20}$	221
SIG055	GCCGGTCTGGTAGTCAAAAC	ATTAGTGTTGGACAAATGCCCC	(AC)$_{9}$	159
SIG056	CTCTTACGTGCATACACAGTTG	AAAGTATGGCCCGGCAGATG	(AC)$_{10}$	151
SIG057	GGGATGAAGTGGTAGAAGAAGC	ATGATGTCCACGGAGAGCC	(AC)$_{13}$	155
SIG058	CCAACCTGCACGCTCTATC	GATCGCGGTCAAAGGTCAAC	(AC)$_{12}$	152
SIG059	CGTACTGATCACCGATGGC	ACCTTTGTACAGCGACCTTG	(AC)$_{12}$	162

[a] Primer pair tested for PCR-amplification in other *Siganus* spp.

[b] Primer pair used for genotyping in *S. luridus, S. rivulatus, S. spinus*, and *S. sutor*

Online Resource 5

GenBank accession numbers for cytochrome *b*-gene sequences of *Siganus* spp.

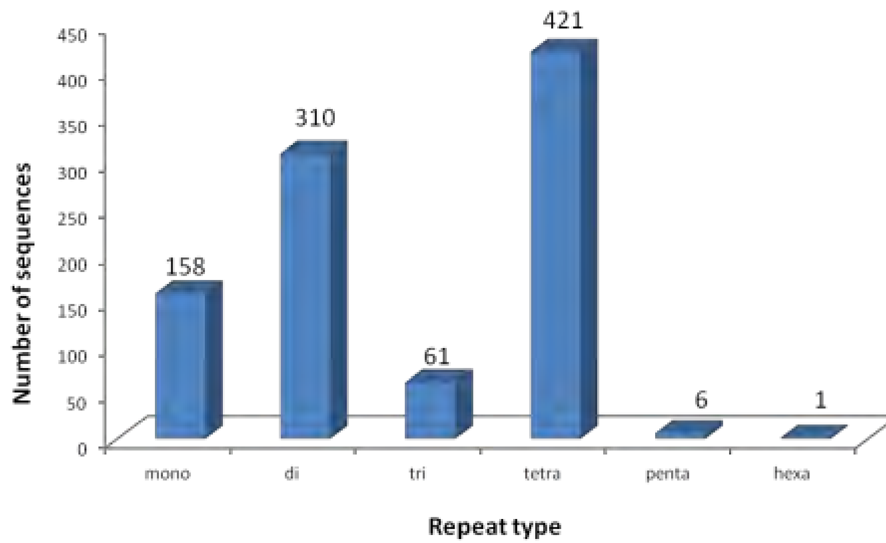
Species	GenBank accession no.
S. argenteus	AB276798-AB276814; AY190542; DQ898028-DQ898036; EF210174
S. canaliculatus	EF210176; GU929677
S. corallinus	AB276925, AB276926, AB276928, AB276930, AB276931
S. doliatus	AB276951-AB276957, AB276960; DQ898038
S. fuscescens	AB276822-AB276825, AB276830; GU929680-GU929684; HQ843182-HQ843185, HQ843187-HQ843191, HQ843196, HQ843197, HQ843200, HQ843201, HQ843209-HQ843217, HQ843227, HQ843228, HQ843230-HQ843232, HQ843235, HQ843236, HQ843238-HQ843242, HQ843246
S. luridus	DQ898056-DQ898058; EF210185, EF210186
S. punctatus	AB276873-AB276880; DQ898068-DQ898074, DQ898092; EF210187
S. puellus	AB276855-AB276867; DQ898063-DQ898067
S. rivulatus	DQ898075-DQ898078
S. spinus	AB276815-AB276821; AY190545, AY190554; DQ898079-DQ898082; EF210189-EF210192
S. sutor	EF210193, EF210194
S. vulpinus	AB276902; AY190548; DQ898084; DQ898085; DQ898086; DQ898087; DQ898088; DQ898089

Some of the sequences available from GenBank were dismissed because of suspected misidentification, mtDNA introgression or cryptic species. These included AB276924-AB276931 labelled *S. corallinus* and AB276834-AB276850, DQ274055, EF210175, GU929678, GU929679 labelled *S. canaliculatus*

Online Resource 6

Cytochrome *b*-gene sequences of *Siganus* spp. that were newly generated, including species name, specimen identification number (ID), sampling location and GenBank number

Species	Specimen ID	Sampling location	GenBank no.
S. laqueus			
	RB09-241	Mozambique	MF326182
	T-66	Seychelles	MF326187
	KEN0001	Kenya	MF326163
	KEN0012	Kenya	MF326164
	KEN0013	Kenya	MF326165
	KEN0030	Kenya	MF326166
	TZW067	Tanzania	MF326188
S. sutor			
	R0301-211	La Réunion	MF326167
	R0301-212	La Réunion	MF326168
	R0301-213	La Réunion	MF326169
	R0301-214	La Réunion	MF326170
	R0301-215	La Réunion	MF326171
	R0301-216	La Réunion	MF326172
	R0301-217	La Réunion	MF326173
	R0301-218	La Réunion	MF326174
	R0301-219	La Réunion	MF326175
	R0301-220	La Réunion	MF326176
	R0301-221	La Réunion	MF326177
	R0301-222	La Réunion	MF326178
	R0301-223	La Réunion	MF326179
	R0301-224	La Réunion	MF326180
	R0301-225	La Réunion	MF326181
	Ssut10	Rodrigues	MF326183
	Ssut14	Rodrigues	MF326184
	Ssut17	Rodrigues	MF326185
	Ssut21	Rodrigues	MF326186



Online Resource 7
Repeat-containing library information for *Siganus sutor***.** Frequency of repeat types in the library (6112 sequences obtained by 454 pyrosequencing). The search criteria for the different repeat types were: ≥10 repeat motifs for mono-nucleotide; ≥8 for di-, tri- and tetra-nucleotide; ≥4 for penta- and hexa-nucleotide.

Online Resource 8

Siganus spp. Species-pairwise Kimura-2-Parameter net mean genetic distance among species, based on cytochrome *b*-gene nucleotide sequences

Species	Species												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *S. argenteus*	-												
2. *S. canaliculatus*	0.164	-											
3. *S. corallinus*	0.140	0.129	-										
4. *S. doliatus*	0.157	0.140	0.011	-									
5. *S. fuscescens*	0.130	0.057	0.144	0.149	-								
6. *S. laqueus*	0.150	0.100	0.092	0.111	0.130	-							
7. *S. luridus*	0.147	0.119	0.178	0.204	0.131	0.156	-						
8. *S. puellus*	1.370	0.105	0.096	0.116	0.136	0.061	0.145	-					
9. *S. punctatus*	1.590	0.110	0.090	0.103	0.134	0.014	0.166	0.064	-				
10. *S. rivulatus*	0.166	0.059	0.176	0.118	0.064	0.127	0.134	0.115	0.131	-			
11. *S. spinus*	0.202	0.120	0.184	0.178	0.088	0.161	0.146	0.177	0.165	0.119	-		
12. *S. sutor*	0.165	0.036	0.161	0.073	0.051	0.111	0.126	0.129	0.128	0.035	0.125	-	
13. *S. vulpinus*	1.132	0.121	0.103	0.116	0.130	0.061	0.141	0.062	0.059	0.128	0.162	0.134	-

Online Resource 9

Pairwise linkage disequilibrium estimates in *S. spinus*

Locus	Locus							
	0RLA2-91	*ORLA11-71*	*ORLA14-42*	*ORLA8-113*	*ORLA12-160*	*SIG006*	*SIG011*	*SIG017*
0RLA2-91	-							
ORLA11-71	0.753	-						
ORLA14-42	0.959	0.792	-					
ORLA8-113	0.998	0.560	0.902	-				
ORLA12-160	0.998	0.346	0.992	0.998	-			
SIG006	0.216	0.691	0.925	0.205	0.186	-		
SIG011	0.620	0.532	0.915	0.107*	0.262*	0.170	-	
SIG017	0.661	0.171	0.923	0.487	0.499	0.455	0.640	-

* $P < 0.05$ (1000 permutations)

Online Resource 10

Pairwise linkage disequilibrium estimates in *Siganus sutor*

Locus	Locus															
	ORLA 2-91	*ORLA 11-71*	*ORLA 14-42*	*ORLA 8-113*	*ORLA 12-160*	*SIG006*	*SIG007*	*SIG008*	*SIG009*	*SIG010*	*SIG011*	*SIG013*	*SIG015*	*SIG017*	*SIG019*	*SIG020*
0RLA2-91	-															
ORLA11-71	0.822															
ORLA14-42	1	0.886	-													
ORLA8-113	0.287	0.620	0.097	-												
ORLA12-160	0.997	0.745	0.994	0.207	-											
SIG006	1	0.514	1	0.199	0.929	-										
SIG007	1	0.998	1	0.231	0.989	1										
SIG008	1	0.736	1	0.192	0.949	0.896	0.998	-								
SIG009	1	0.990	1	0.941	1	1*	1	1	-							
SIG010	1	0.997	1	0.524	1	1	1	1	1*	-						
SIG011	1	0.979	1	0.353	0.999	1	1	0.785	1*	1*	-					
SIG013	0.986	0.343	0.859	0.770	0.555	0.260	0.537	0.091	1	0.995	0.511	-				
SIG015	1	0.769	1	0.230	0.991	1	1	1	1	1	1	0.900	-			
SIG017	0.915	0.271	0.734	0.452	0.634	1	0.970	0.990	1	0.953	0.776	0.921	0.933	-		
SIG019	1	0.831	1	0.604	0.998	1	1	0.999	1	1	1	0.904	1	0.954	-	
SIG020	1	0.437	1	0.425	0.999	0.889	1	0.615	1*	1	0.977	0.972	1	0.502	0.954	-

* $P < 0.05$ (1000 permutations)