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Hoareau

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Ilkser Erdem Kiper, Paulette Bloomer, Philippe Borsa, Thierry Bernard Hoareau. Characterization of genome-wide microsatellite markers in rabbitfishes, an important resource for artisanal fisheries in the Indo-West Pacific. *Molecular Biology Reports*, 2018, 45 (1), pp.19-25. 10.1007/s11033-017-4136-x . ird-01700836

**HAL Id: ird-01700836**

**<https://ird.hal.science/ird-01700836>**

Submitted on 5 Feb 2018

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*To be cited as:*

*Kiper IE, Bloomer P, Borsa P, Hoareau TB (2018) Characterization of genome-wide microsatellite markers in rabbitfishes, an important resource for artisanal fisheries in the Indo-West Pacific. Molecular Biology Reports 45, 19-25. , doi: 10.1007/s11033-017-4136-x*

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

Ilkser Erdem Kiper <sup>1,2</sup>, Paulette Bloomer <sup>1,2</sup>, Philippe Borsa <sup>3</sup>, Thierry Bernard Hoareau <sup>1</sup> <sup>2</sup>

<sup>1</sup> Molecular Ecology and Evolution Programme, Department of Genetics University of Pretoria Private Bag X20, Hatfield, Pretoria 0028, South Africa

<sup>2</sup> Department of Genetics, University of Pretoria, Private Bag X20 Hatfield 0028 South Africa

<sup>3</sup> UMR 250 “Ecologie marine tropicale des océans Pacifique et Indien”, Institut de recherche pour le développement (IRD), BP A5, 98848 Nouméa, New Caledonia

 Molecular Ecology and Evolution Programme, Department of Genetics, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa; Phone: +27(0)12 420 5062; Email: thoareau@gmail.com

Received: 23 September 2016 / Accepted: 12 December 2017

**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. spinus*, *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<sub>6</sub>, GAAA<sub>6</sub>, and GATA<sub>6</sub>) [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  $\mu$ L volumes and the reaction mixture included  $\sim$  40 ng of template DNA, 1 $\times$  PCR buffer, 1.5 mM MgCl<sub>2</sub>, 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  $\mu$ L 70% ethanol. The products were eluted in 15  $\mu$ L Sabax water. Cycle sequencing reactions contained approximately 30 ng purified DNA, 1  $\mu$ L 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 ( $t = 5\text{--}300$ ) 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 and 14 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.

## Acknowledgements

We would like to thank the following collaborators for facilitating the collection of samples: A. Brito and R. Mutombene from the Instituto Nacional de Investigaçao Pesqueira (Mozambique), N. Wambiji from the Marine & Fisheries Research Institute (Kenya), S. Abeare from Reef Doctor (Madagascar), G. Gouws from the South African Institute for Aquatic Biodiversity (South Africa), A. Collet, G. Mou-Tham and D. Lecchini from IRD (New Caledonia), P. Boblin and E. Tardy from SPC (New Caledonia), E. Tessier from Parc Marin (La Réunion), F. Giancarlo from Proyek Biokor (Indonesia and West Papua), M. Hassan from SMEL (France), and M. Low from NUS (Singapore).

## Funding

This project was funded by the Western Indian Ocean Marine Science Association (WIOMSA): MARG I/11/2013 and the South African Department of Science and Technology/National Research Foundation African Coelacanth Ecosystem Programme (ACEP) II. Sampling was funded in part by IRD, SPC and SAIAB. IEK benefitted from a PhD scholarship from the University of Pretoria's PhD programme. TBH was supported by the University of Pretoria's research fellowship programme and the University of Pretoria Research Development Programme.

## 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                |                         |                       |                      |
|------------|-----------|------------------------|-------------------------|-----------------------|----------------------|
|            |           | <i>S. luridus</i> (17) | <i>S. rivulatus</i> (7) | <i>S. spinus</i> (20) | <i>S. sutor</i> (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     |           |                        |                         |                       |                      |

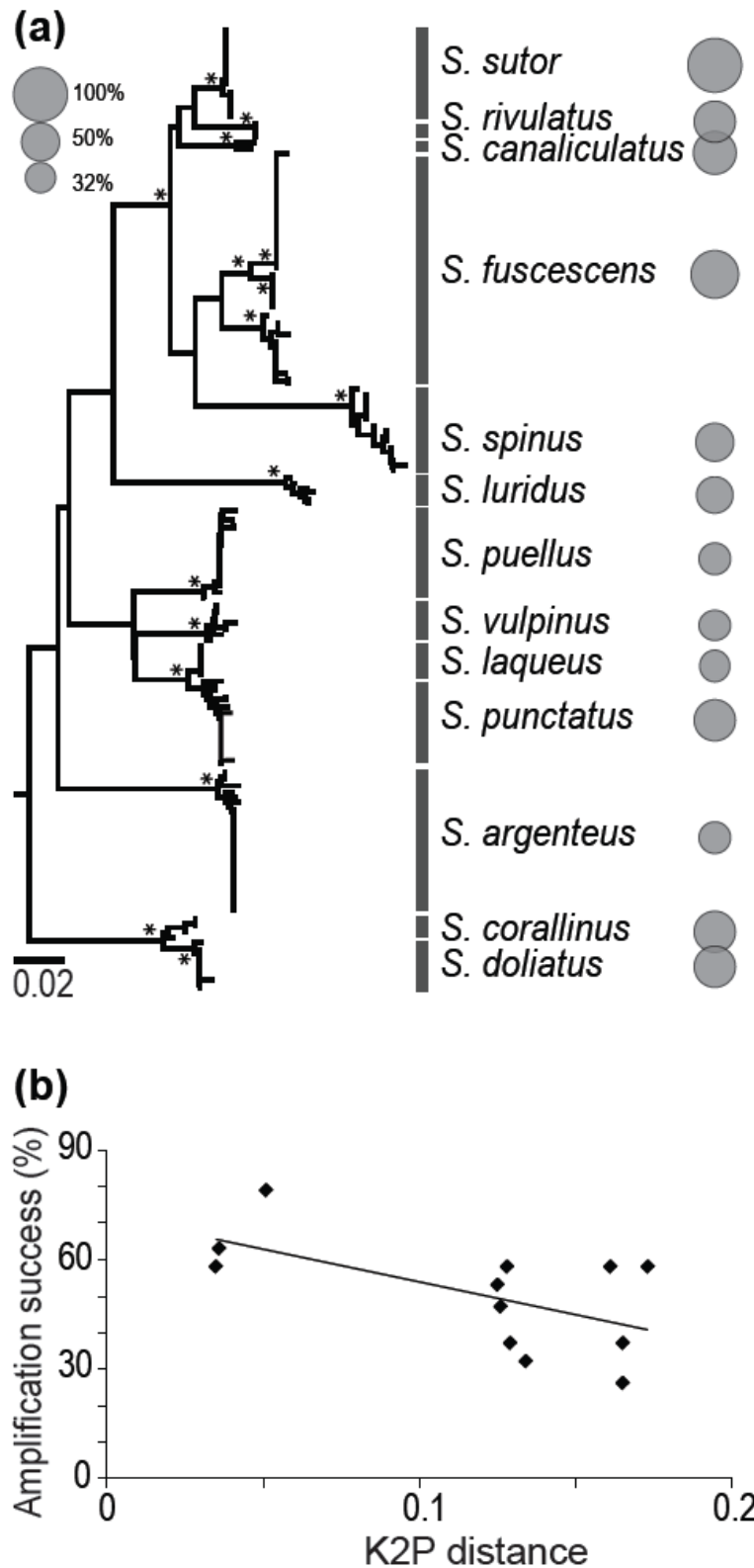
|               |          |        |        |       |          |
|---------------|----------|--------|--------|-------|----------|
| <i>SIG014</i> | $N_A$    | MD     | NA     | MD    | 5        |
|               | $H_E$    |        |        |       | 0.658    |
|               | $H_O$    |        |        |       | 0.044    |
|               | $F_{IS}$ |        |        |       | 0.935*** |
| <i>SIG015</i> | $N_A$    | 12     | 8      | MD    | MD       |
|               | $H_E$    | 0.909  | 0.912  |       |          |
|               | $H_O$    | 0.688  | 0.714  |       |          |
|               | $F_{IS}$ | 0.250* | 0.231  |       |          |
| <i>SIG017</i> | $N_A$    | NA     | NA     | NA    | 15       |
|               | $H_E$    |        |        |       | 0.899    |
|               | $H_O$    |        |        |       | 0.781    |
|               | $F_{IS}$ |        |        |       | 0.133*   |
| <i>SIG019</i> | $N_a$    | 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    |
| <i>SIG020</i> | $N_A$    | NA     | NA     | MD    | 12       |
|               | $H_E$    |        |        |       | 0.806    |
|               | $H_O$    |        |        |       | 0.781    |
|               | $F_{IS}$ |        |        |       | 0.031    |
|               | $N_A$    | 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    |

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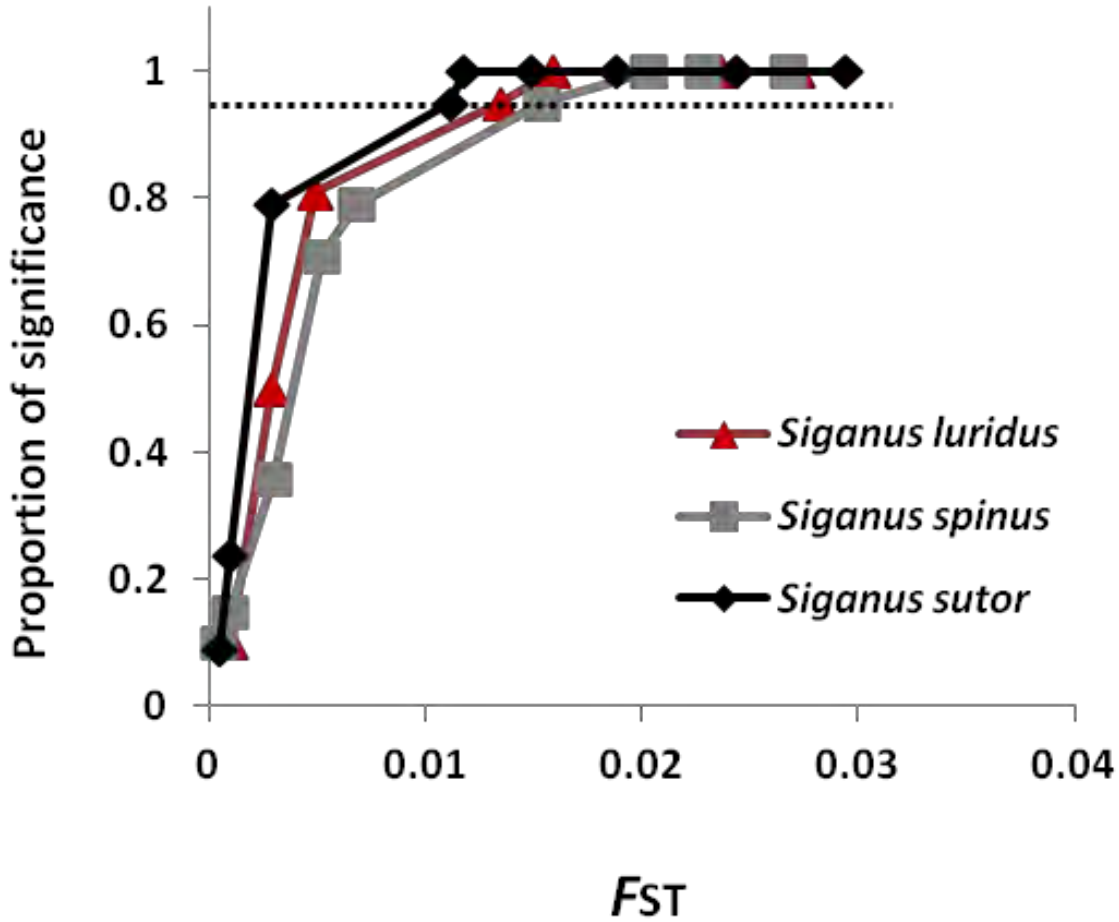
\*  $P < 0.05$ ; \*\*\*  $P < 0.001$ . *NA*: locus with no amplification, *MD*: locus with missing data (a proportion of the individuals were not amplified)

**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 |
|-------------------|---------------------|----|-------|------------------|------------|
| <i>S. luridus</i> | Among populations   | 2  | 15.19 | $F_{ST} : 0.152$ | 0.001      |
|                   | Within populations  | 31 | 84.81 |                  |            |
| <i>S. spinus</i>  | 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 ( $\geq 75\%$  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;  $PS = 2.076 + 0.261 \cdot \ln(F_{ST})$ ;  $F_{ST95} = 0.0135$ ), *S. spinus* (8 loci; 20 individuals;  $PS = 2.055 + 0.265 \cdot \ln(F_{ST})$ ;  $F_{ST95} = 0.015$ ), and *S. sutor* (16 loci; 32 individuals;  $PS = 1.991 + 0.232 \cdot \ln(F_{ST})$ ;  $F_{ST95} = 0.011$ ). 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**

Ilkser Erdem Kiper, Paulette Bloomer, Philippe Borsa, Thierry Bernard Hoareau

Correspondence: Molecular Ecology and Evolution Programme, Department of Genetics, University of Pretoria, Pretoria 0002, South Africa

[thoareau@gmail.com](mailto:thoareau@gmail.com)

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   |
|-------------------------|--------------------------|---------------|-----------------------------------------------------------|-------------|
| <i>S. argenteus</i>     | IFA0001                  | 11 Dec. 2013  | Mangily, Ifaty, Madagascar                                | S. Abeare   |
|                         | S006                     | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
| <i>S. canaliculatus</i> | 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    |
| <i>S. corallinus</i>    | T-392                    | 30 Apr. 2005  | SE side, Conception Is., Seychelles                       | G. Gouws    |
|                         | S002<br>(MNHN 2007-0006) | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
| <i>S. doliatus</i>      | S003<br>(MNHN 2007-0007) | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
|                         | S007<br>(MNHN 2007-0008) | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
| <i>S. fuscescens</i>    | Sfus04                   | June 2006     | Marau, Solomon Islands,                                   | E. Tardy    |
|                         | Sfus05                   | June 2006     | Marau, Solomon Islands,                                   | E. Tardy    |
| <i>S. laqueus</i>       | 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  |
| <i>S. luridus</i>       | 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 |
| <i>S. puellus</i>       | S004                     | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
| <i>S. punctatus</i>     | S001<br>(MNHN 2007-0010) | 04 Feb. 2005  | Nouméa fish market, New Caledonia                         | A. Collet   |
| <i>S. rivulatus</i>     | 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   |
| <i>S. spinus</i>        | 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   |

|                    |            |              |                                   |              |
|--------------------|------------|--------------|-----------------------------------|--------------|
| <i>S. sutor</i>    | 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  |
|                    | INS0001    | 31 Mar. 2014 | Inhassoro, Mozambique             | A. Brito     |
|                    | INS0002    | 31 Mar. 2014 | Inhassoro, Mozambique             | A. Brito     |
|                    | INS0004    | 31 Mar. 2014 | Inhassoro, Mozambique             | A. Brito     |
| <i>S. vulpinus</i> | 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     |
|                    | 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               |

<sup>a</sup> 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) |
|-----------------------------------|-------|------------------------------------------------|------------|-------------------|
| <i>ORLA12-91</i> <sup>b, c</sup>  | NED   | (GT) <sub>7</sub>                              | 60         | 134-174           |
| <i>ORLA8-113</i> <sup>b, c</sup>  | PET   | (CA) <sub>13</sub>                             | 60         | 161-167           |
| <i>ORLA11-71</i> <sup>a, c</sup>  | 6-FAM | (CA) <sub>8</sub>                              | 60         | 377-387           |
| <i>ORLA12-160</i> <sup>b, c</sup> | PET   | (CA) <sub>12</sub> (TA)(CA) <sub>6</sub>       | 60         | 283-303           |
| <i>ORLA14-42</i> <sup>b, c</sup>  | PET   | (CA) <sub>10</sub>                             | 60         | 368-400           |
| <i>SIG006</i> <sup>a</sup>        | NED   | (CAAA) <sub>9</sub>                            | 55         | 215-251           |
| <i>SIG007</i> <sup>a</sup>        | VIC   | (CAAA) <sub>5</sub> (CAGA)(CAAA) <sub>8</sub>  | 55         | 191-259           |
| <i>SIG008</i> <sup>a</sup>        | 6-FAM | (ATAG) <sub>4</sub> (GTAG)(ATAG) <sub>11</sub> | 55         | 130-186           |
| <i>SIG009</i> <sup>a</sup>        | PET   | (AGAT) <sub>18</sub>                           | 55         | 196-352           |
| <i>SIG010</i> <sup>a</sup>        | 6-FAM | (AGAT) <sub>12</sub>                           | 55         | 238-282           |
| <i>SIG011</i> <sup>b</sup>        | VIC   | (AGAT) <sub>16</sub>                           | 55         | 184-268           |
| <i>SIG013</i> <sup>b</sup>        | 6-FAM | (GTTT) <sub>11</sub>                           | 57         | 266-282           |
| <i>SIG014</i> <sup>a</sup>        | VIC   | (GTTT) <sub>11</sub>                           | 55         | 333-369           |
| <i>SIG015</i> <sup>b</sup>        | NED   | (CTTT) <sub>11</sub> (CTCT)(CTTT) <sub>6</sub> | 55         | 243-299           |
| <i>SIG017</i> <sup>a</sup>        | PET   | (CAG) <sub>8</sub>                             | 55         | 102-117           |
| <i>SIG019</i> <sup>b</sup>        | 6-FAM | (GTT) <sub>12</sub>                            | 55         | 187-235           |
| <i>SIG020</i> <sup>a</sup>        | NED   | (ACC) <sub>15</sub>                            | 57         | 115-157           |

<sup>a</sup> Multiplex combination 1

<sup>b</sup> Multiplex combination 2

<sup>c</sup> 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) |
|-------------------------------|------------------------|------------------------|------------------------------------------------|--------------------|
| <i>SIG001</i>                 | GTGGCACGAACAACACATGA   | GGGCTTTGCAAGTCACGAAGTC | (ATCT) <sub>10</sub>                           | 231                |
| <i>SIG002</i> <sup>a</sup>    | CTTGGCAAGCAATGGAAGC    | AGTGAGTTGCCTGGAAAG     | (ATCT) <sub>21</sub>                           | 308                |
| <i>SIG003</i> <sup>a</sup>    | ACATTTAGCGTGGGAGAC     | TTCTGCATCAGACTGAAC     | (ATCT) <sub>12</sub>                           | 153                |
| <i>SIG004</i> <sup>a</sup>    | CCTCCGTGGAAGTCAACAG    | AATCATGGCGACTGTTGAGC   | (GT) <sub>9</sub>                              | 175                |
| <i>SIG005</i> <sup>a</sup>    | GTCGAAGGTGCTAGTTCTTG   | GCATGCCTGTGACTGTCAAC   | (ATCT) <sub>13</sub>                           | 261                |
| <i>SIG006</i> <sup>a, b</sup> | AACTGTGTGAGCGATCTG     | TCAGTACAGCGTGACATC     | (CAAA) <sub>9</sub>                            | 237                |
| <i>SIG007</i> <sup>a, b</sup> | AAACAAACGTCCGGAAC      | GGTCAGTCAGCATCGTAA     | (CAAA) <sub>5</sub> (CAGA)(CAAA) <sub>8</sub>  | 265                |
| <i>SIG008</i> <sup>a, b</sup> | TGCTAAATGTCCCTTCAC     | TGGATCTGTGCTACCTAC     | (ATAG) <sub>4</sub> (GTAG)(ATAG) <sub>11</sub> | 160                |
| <i>SIG009</i> <sup>a, b</sup> | TCCTGCTACCCACTCCTTC    | ATTACAGGAAGCGGAAAC     | (AGAT) <sub>18</sub>                           | 243                |
| <i>SIG010</i> <sup>a, b</sup> | ATCTGACGGATACGATTC     | TCGAGCAGAATGGCACTCAG   | (AGAT) <sub>12</sub>                           | 269                |
| <i>SIG011</i> <sup>a, b</sup> | TTCTCCTAGGCCTGGAGATG   | CCTCCTCTAATGCACCTAT    | (AGAT) <sub>16</sub>                           | 211                |
| <i>SIG012</i> <sup>a</sup>    | GGCTCGTTGACCTCTCAG     | AGAACAACTCCGGAAC       | (GTTT) <sub>8</sub>                            | 293                |
| <i>SIG013</i> <sup>a, b</sup> | CGGCTCTTTTGTGTCTGT     | ACAATGTTGCGCTCACTC     | (GTTT) <sub>11</sub>                           | 269                |
| <i>SIG014</i> <sup>a, b</sup> | TGGCTGTTCTTACTGTCT     | TATGACGGCCACTAGATG     | (GTTT) <sub>11</sub>                           | 354                |
| <i>SIG015</i> <sup>a, b</sup> | GGGGAGAGAAATGATAGC     | CCGCATTAGTAAATTGCTC    | (CTTT) <sub>11</sub> (CTCT)(CTTT) <sub>6</sub> | 279                |
| <i>SIG016</i> <sup>a</sup>    | GGCTATGCACTGATTAC      | ACATAATCCATCAGCAG      | (AGC) <sub>8</sub>                             | 124                |
| <i>SIG017</i> <sup>a, b</sup> | GAGTGGGTGTCTCTGAATG    | AGCATCAATAGACGTGTG     | (CAG) <sub>8</sub>                             | 111                |
| <i>SIG018</i> <sup>a</sup>    | ACTGATGAGCTGGTTTTTC    | TGCTGCTGTCAGCAGTTC     | (AGG) <sub>9</sub>                             | 115                |
| <i>SIG019</i> <sup>a, b</sup> | AAGTAGCGCCACCTTCTG     | TGACAGCTTGCAGCAGAA     | (GTT) <sub>12</sub>                            | 198                |
| <i>SIG020</i> <sup>a, b</sup> | TCTGAACCATGAGCTTCC     | TCGAACGGAGACGCTTCA     | (ACC) <sub>15</sub>                            | 138                |
| <i>SIG021</i>                 | CTTCTCGCCTTCCAGCTCATC  | TGTGATGAGTACCTGAGTAA   | (CTTT) <sub>10</sub>                           | 349                |
| <i>SIG022</i>                 | GAGGCAGTGACAGCCTGTCT   | GTAAGGTGCTGCAGCAGGATG  | (CTTT) <sub>14</sub>                           | 189                |
| <i>SIG023</i>                 | ACACAGGACCTGCAAGAGA    | TGCGGATAGGCTCCAATGACC  | (ATCT) <sub>14</sub>                           | 268                |
| <i>SIG024</i>                 | ATGAGACCGTACATCTTCATC  | TTGTTGGGTTTAGTTCTAGGA  | (GT) <sub>13</sub>                             | 173                |
| <i>SIG025</i>                 | CCAATGCAGCACATTCACT    | GGATGTTGTAGCAGCCTCCA   | (GT) <sub>11</sub>                             | 130                |
| <i>SIG026</i>                 | TTTCCTCTACCACCTCTCATC  | CGAATGACTTCGCTGGAAC    | (ATCT) <sub>10</sub>                           | 223                |
| <i>SIG027</i>                 | TCAGAACCAGCTCTCACTGC   | AAGAAGTACGAGTGATAGAG   | (CT) <sub>9</sub>                              | 163                |
| <i>SIG028</i>                 | GGGGGCGCAATAGTAAGATG   | TCAGATTGGAGGCCACAATCC  | (ATCT) <sub>19</sub>                           | 245                |
| <i>SIG029</i>                 | TCCTCTGCCATTAGGTTCTG   | GGCGTAAACTTACAGTACA    | (GT) <sub>9</sub>                              | 150                |
| <i>SIG030</i>                 | TTATGCCACACTGGAGGTTT   | CCTCCCTTGATGTGGAGTAG   | (GT) <sub>11</sub>                             | 160                |
| <i>SIG031</i>                 | CCACAACACACATATACAC    | GTGCAGCAGTAAGGTGGTCT   | (ATCT) <sub>15</sub>                           | 204                |
| <i>SIG032</i>                 | F:GAGGGCTTCTCTTACAGTAG | ATGGAGGCGAGAGGCAGAGA   | (ATCT) <sub>19</sub>                           | 294                |
| <i>SIG033</i>                 | CATCAACAGGTTGGCTACAG   | GTACCAGCCTGGAACTAGC    | (GT) <sub>8</sub>                              | 156                |
| <i>SIG034</i>                 | TCCTCATCGCACTCGCCAGA   | ACGCAGATCGTGATGAGTC    | (ATCT) <sub>13</sub>                           | 300                |
| <i>SIG035</i>                 | GGCAGTGCTGGTGGATCTAC   | ACGCAGAGGAAACACAGAAC   | (GT) <sub>9</sub>                              | 152                |
| <i>SIG036</i>                 | GTGGCCGTTCTTATATTATG   | CTAATGAGGTCGAGTCC      | (GT) <sub>8</sub>                              | 136                |
| <i>SIG037</i>                 | CAGCGCTGATGGCACAATCC   | CTTCTCTCCTTTTCTCTAC    | (GT) <sub>18</sub>                             | 224                |
| <i>SIG038</i>                 | GGATGAAAACATCGGAGTC    | GACTGCACAACACAGCTATGG  | (AGAT) <sub>15</sub>                           | 193                |

|        |                          |                          |                      |     |
|--------|--------------------------|--------------------------|----------------------|-----|
| SIG039 | GTGGGGCCAATATCTGTGGG     | AGCCTGGCCAATATCTGAGTG    | (ATCT) <sub>12</sub> | 208 |
| SIG040 | TGACGACTAGGCTAGCAGAG     | ACCTCAGGCTCCAATACCTT     | (AAAC) <sub>13</sub> | 194 |
| SIG041 | CTGGATGCACAGGTGTGTA      | GGTTCACAGGTGGCTGCTGTG    | (AAAC) <sub>13</sub> | 236 |
| SIG042 | CTGGGTCGAGTCGCCCCC       | TGCTTCTGTCGTTGCATCAG     | (AAAC) <sub>10</sub> | 166 |
| SIG043 | ACCTGCCATCTGTATCATTC     | CATGGCGGGAAACGTTCTGAGC   | (AC) <sub>8</sub>    | 122 |
| SIG044 | CGTAGCCCGTGGCCGTGTAG     | CGTTAGCCCGTCCACGGCGT     | (AC) <sub>11</sub>   | 164 |
| SIG045 | AGTGTATGAGGACGAGCGG      | TGTTGTCTCACTTTACAACAACAC | (AAAC) <sub>9</sub>  | 236 |
| SIG046 | TCAACAACCTGGAATCAACAACTG | CTGATCTCCGTGCTCCACC      | (AAAC) <sub>13</sub> | 248 |
| SIG047 | AGAAACAAACGTCCGGAAAC     | CCTGAGTAATCATCTCCGGC     | (AAAC) <sub>8</sub>  | 311 |
| SIG048 | TGACTCATGCACAAGTACATCC   | TTGTGGTAATATACAGGGCGG    | (AAAC) <sub>13</sub> | 187 |
| SIG049 | ACACACTCCCTATTCCTGC      | TAACGTCTGGGACGAGAGC      | (AGAT) <sub>17</sub> | 197 |
| SIG050 | TCACGCTCAGCAAGTAACC      | ACTGGTGAGACTATTCTTGCC    | (AAAC) <sub>10</sub> | 176 |
| SIG051 | ACTGTTGCCATAGTGAAGTGC    | AGCGCACTGTGTCCATTAG      | (AC) <sub>8</sub>    | 161 |
| SIG052 | ACATTTATGTCTTAGCAACGGGG  | ACAAAGTGGAGGGCAACTG      | (AC) <sub>11</sub>   | 181 |
| SIG053 | TCCCCACAGTGTTCACC        | ACAGCTTTTGCCCTTCAG       | (AC) <sub>10</sub>   | 232 |
| SIG054 | ACACTCTCACACAGACCTGC     | ACGGCGAGCCTACTTACTTC     | (AC) <sub>20</sub>   | 221 |
| SIG055 | GCCGGTCTGGTAGTCAAAAC     | ATTAGTGTGGACAAATGCCCC    | (AC) <sub>9</sub>    | 159 |
| SIG056 | CTCTTACGTGCATACAGTTG     | AAAGTATGGCCCGGCAGATG     | (AC) <sub>10</sub>   | 151 |
| SIG057 | GGGATGAAGTGGTAGAAGAAGC   | ATGATGTCCACGGAGAGCC      | (AC) <sub>13</sub>   | 155 |
| SIG058 | CCAACCTGCACGCTCTATC      | GATCGCGGTCAAAGGTCAAC     | (AC) <sub>12</sub>   | 152 |
| SIG059 | CGTACTGATCACCATGGC       | ACCTTTGTACAGCGACCTTG     | (AC) <sub>12</sub>   | 162 |

<sup>a</sup> Primer pair tested for PCR-amplification in other *Siganus* spp.

<sup>b</sup> 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.

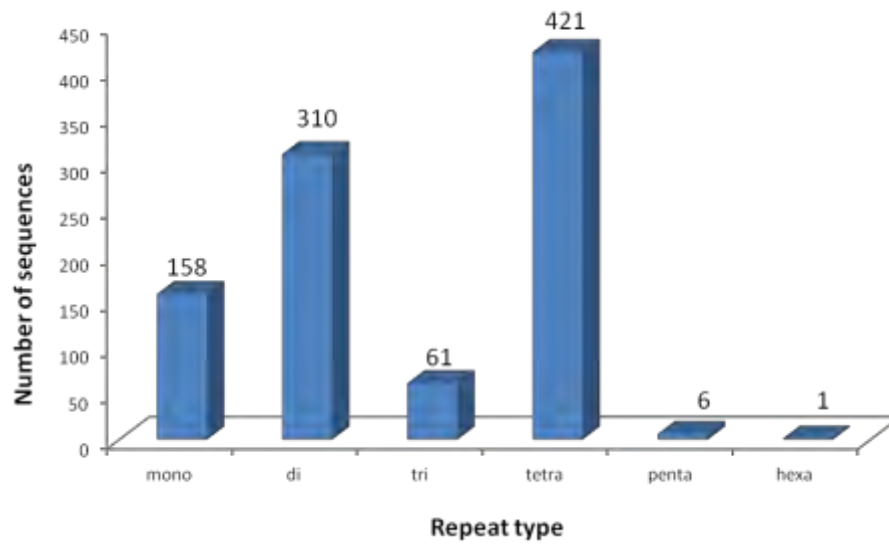
| Species                 | GenBank accession no.                                                                                                                                                                                                                   |
|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>S. argenteus</i>     | AB276798-AB276814; AY190542; DQ898028-DQ898036; EF210174                                                                                                                                                                                |
| <i>S. canaliculatus</i> | EF210176; GU929677                                                                                                                                                                                                                      |
| <i>S. corallinus</i>    | AB276925, AB276926, AB276928, AB276930, AB276931                                                                                                                                                                                        |
| <i>S. doliatus</i>      | AB276951-AB276957, AB276960; DQ898038                                                                                                                                                                                                   |
| <i>S. fuscescens</i>    | AB276822-AB276825, AB276830; GU929680-GU929684; HQ843182-HQ843185, HQ843187-HQ843191, HQ843196, HQ843197, HQ843200, HQ843201, HQ843209-HQ843217, HQ843227, HQ843228, HQ843230-HQ843232, HQ843235, HQ843236, HQ843238-HQ843242, HQ843246 |
| <i>S. luridus</i>       | DQ898056-DQ898058; EF210185, EF210186                                                                                                                                                                                                   |
| <i>S. punctatus</i>     | AB276873-AB276880; DQ898068-DQ898074, DQ898092; EF210187                                                                                                                                                                                |
| <i>S. puellus</i>       | AB276855-AB276867; DQ898063-DQ898067                                                                                                                                                                                                    |
| <i>S. rivulatus</i>     | DQ898075-DQ898078                                                                                                                                                                                                                       |
| <i>S. spinus</i>        | AB276815-AB276821; AY190545, AY190554; DQ898079-DQ898082; EF210189-EF210192                                                                                                                                                             |
| <i>S. sutor</i>         | EF210193, EF210194                                                                                                                                                                                                                      |
| <i>S. vulpinus</i>      | 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. |
|-------------------|-------------|-------------------|-------------|
| <i>S. laqueus</i> | RB09-241    | Mozambique        | MF326182    |
|                   | T-66        | Seychelles        | MF326187    |
|                   | KEN0001     | Kenya             | MF326163    |
|                   | KEN0012     | Kenya             | MF326164    |
|                   | KEN0013     | Kenya             | MF326165    |
|                   | KEN0030     | Kenya             | MF326166    |
|                   | TZW067      | Tanzania          | MF326188    |
| <i>S. sutor</i>   | 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:  $\geq 10$  repeat motifs for mono-nucleotide;  $\geq 8$  for di-, tri- and tetra-nucleotide;  $\geq 4$  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. <i>S. argenteus</i>     | -       |       |       |       |       |       |       |       |       |       |       |       |    |
| 2. <i>S. canaliculatus</i> | 0.164   | -     |       |       |       |       |       |       |       |       |       |       |    |
| 3. <i>S. corallinus</i>    | 0.140   | 0.129 | -     |       |       |       |       |       |       |       |       |       |    |
| 4. <i>S. doliatus</i>      | 0.157   | 0.140 | 0.011 | -     |       |       |       |       |       |       |       |       |    |
| 5. <i>S. fuscescens</i>    | 0.130   | 0.057 | 0.144 | 0.149 | -     |       |       |       |       |       |       |       |    |
| 6. <i>S. laqueus</i>       | 0.150   | 0.100 | 0.092 | 0.111 | 0.130 | -     |       |       |       |       |       |       |    |
| 7. <i>S. luridus</i>       | 0.147   | 0.119 | 0.178 | 0.204 | 0.131 | 0.156 | -     |       |       |       |       |       |    |
| 8. <i>S. puellus</i>       | 1.370   | 0.105 | 0.096 | 0.116 | 0.136 | 0.061 | 0.145 | -     |       |       |       |       |    |
| 9. <i>S. punctatus</i>     | 1.590   | 0.110 | 0.090 | 0.103 | 0.134 | 0.014 | 0.166 | 0.064 | -     |       |       |       |    |
| 10. <i>S. rivulatus</i>    | 0.166   | 0.059 | 0.176 | 0.118 | 0.064 | 0.127 | 0.134 | 0.115 | 0.131 | -     |       |       |    |
| 11. <i>S. spinus</i>       | 0.202   | 0.120 | 0.184 | 0.178 | 0.088 | 0.161 | 0.146 | 0.177 | 0.165 | 0.119 | -     |       |    |
| 12. <i>S. sutor</i>        | 0.165   | 0.036 | 0.161 | 0.073 | 0.051 | 0.111 | 0.126 | 0.129 | 0.128 | 0.035 | 0.125 | -     |    |
| 13. <i>S. vulpinus</i>     | 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           |                  |                  |                  |                   |               |               |               |
|-------------------|-----------------|------------------|------------------|------------------|-------------------|---------------|---------------|---------------|
|                   | <i>ORLA2-91</i> | <i>ORLA11-71</i> | <i>ORLA14-42</i> | <i>ORLA8-113</i> | <i>ORLA12-160</i> | <i>SIG006</i> | <i>SIG011</i> | <i>SIG017</i> |
| <i>ORLA2-91</i>   | -               |                  |                  |                  |                   |               |               |               |
| <i>ORLA11-71</i>  | 0.753           | -                |                  |                  |                   |               |               |               |
| <i>ORLA14-42</i>  | 0.959           | 0.792            | -                |                  |                   |               |               |               |
| <i>ORLA8-113</i>  | 0.998           | 0.560            | 0.902            | -                |                   |               |               |               |
| <i>ORLA12-160</i> | 0.998           | 0.346            | 0.992            | 0.998            | -                 |               |               |               |
| <i>SIG006</i>     | 0.216           | 0.691            | 0.925            | 0.205            | 0.186             | -             |               |               |
| <i>SIG011</i>     | 0.620           | 0.532            | 0.915            | 0.107*           | 0.262*            | 0.170         | -             |               |
| <i>SIG017</i>     | 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<br>2-91 | ORLA<br>11-71 | ORLA<br>14-42 | ORLA<br>8-113 | ORLA<br>12-160 | SIG006 | SIG007 | SIG008 | SIG009 | SIG010 | SIG011 | SIG013 | SIG015 | SIG017 | SIG019 | SIG020 |
| ORLA2-91   | -            |               |               |               |                |        |        |        |        |        |        |        |        |        |        |        |
| 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)