

## Characterization of 11 novel microsatellite markers for the vagabond butterflyfish, *Chaetodon vagabundus*

Item Type	Article
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Citation	Characterization of 11 novel microsatellite markers for the vagabond butterflyfish, <i>Chaetodon vagabundus</i> 2015, 7 (3):713 Conservation Genetics Resources
Eprint version	Post-print
DOI	<a href="https://doi.org/10.1007/s12686-015-0440-8">10.1007/s12686-015-0440-8</a>
Publisher	Springer Nature
Journal	Conservation Genetics Resources
Rights	Archived with thanks to Conservation Genetics Resources. The final publication is available at Springer via <a href="http://dx.doi.org/10.1007/s12686-015-0440-8">http://dx.doi.org/10.1007/s12686-015-0440-8</a>
Download date	2024-03-13 09:38:16
Link to Item	<a href="http://hdl.handle.net/10754/570790">http://hdl.handle.net/10754/570790</a>

**Conservation Genetics Resources**  
**Characterization of 11 novel microsatellite markers for the vagabond butterflyfish,  
*Chaetodon vagabundus***  
--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Full Title:</b>	Characterization of 11 novel microsatellite markers for the vagabond butterflyfish, <i>Chaetodon vagabundus</i>
<b>Article Type:</b>	Microsatellite Letters
<b>Keywords:</b>	primer development, coral reef fish, 454 pyrosequencing, connectivity
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<b>Abstract:</b>	Microsatellite markers were developed for the coral reef fish <i>Chaetodon vagabundus</i> using shotgun pyrosequencing. As threats to coral reefs intensify, information on larval connectivity is of increasing value for efficient conservation planning. Here, 11 novel microsatellites were characterized for 192 individuals from Papua New Guinea. The number of alleles per locus ranged from 7 to 32, while observed and expected heterozygosity values varied from 0.214 to 0.903. These markers will be used to study population structure and larval connectivity of this iconic coral reef fish in coral reefs across the Indo-Pacific.
<b>Suggested Reviewers:</b>	

14 January 2015  
Editorial Office  
Conservation Genetic Resources

Dear Editor,

We are submitting the manuscript “**Characterization of 11 novel microsatellite markers for the vagabond butterflyfish, *Chaetodon vagabundus***” for consideration as *a microsatellite letter* in Conservation Genetic Resources.

The manuscript describes the development of genetic markers for an iconic coral reef fish. As threats to coral reefs intensify, information on larval connectivity is of increasing value for efficient conservation planning. These markers will be used to study population structure and larval connectivity of this iconic coral reef fish in coral reefs across the Indo-Pacific.

As requested, the sequences of these microsatellites will be submitted as electronic supplementary material. We would like to point out that Gene bank accession number in Table one has been provisionally and temporally left blank. If the manuscript is accepted, we will proceed to upload the sequences to Genbank. In this way we would make sure the sequences have the appropriate reference as part of their metadata. Of course, we would be happy to proceed otherwise if you believe it should be the case.

All the authors have agreed to this version of the manuscript, and we certify that this manuscript, or parts of it, have not been published elsewhere and are not under consideration for publishing elsewhere. On behalf of all my co-authors, I look forward to hearing from you.

Yours sincerely,

Pablo Saenz-Agudelo

## **Characterization of 11 novel microsatellite markers for the vagabond butterflyfish, *Chaetodon vagabundus***

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Keywords: primer development, coral reef fish, 454 pyrosequencing, connectivity

### **Abstract**

Microsatellite markers were developed for the coral reef fish *Chaetodon vagabundus* using shotgun pyrosequencing. As threats to coral reefs intensify, information on larval connectivity is of increasing value for efficient conservation planning. Here, 11 novel microsatellites were characterized for 192 individuals from Papua New Guinea. The number of alleles per locus ranged from 7 to 32, while observed and expected heterozygosity values varied from 0.214 to 0.903. These markers will be used to study population structure and larval connectivity of this iconic coral reef fish in coral reefs across the Indo-Pacific.

In recent decades, coral reefs have faced increasing pressures, resulting in dramatic decreases in coral cover on coral reefs worldwide (e.g. De'ath et al. 2012). Marine protected areas (MPAs) networks have been advocated as conservation alternatives to preserve coral reefs, but their effectiveness relies heavily on the extent to which individual MPAs are connected, primarily via larval dispersal (Green et al. 2014).

*Cheatodon vagabundus*, a member of the iconic family of butterflyfishes (Chaetodontidae), is common on Indo-Pacific coral reefs. Adults release gametes directly into the water column; larvae spend around 38 days in the pelagic environment (Almany et al. 2007) and then recruit to coral reefs where they will spend the rest of their lives. This reproductive cycle is typical of the majority of marine fishes and most species targeted by fisheries.. Thus, a deeper understanding of the patterns of population connectivity of this species will provide valuable information that could be used to optimize size and spacing of MPAs in coral reefs worldwide (e.g. Berumen et al. 2012).

Here we characterized 11 novel microsatellite markers in *C. vagabundus*, increasing the number of microsatellites already available for the species (Almany et al. 2009). These loci were tested on 192 individuals from Kimbe Bay, Papua New Guinea. Genomic DNA was extracted from fin clip tissues using the Qiagen DNeasy blood and tissue kit according to the manufacturer's protocol. Library preparation and 454 GS FLX titanium shotgun sequencing was performed on one individual collected in the same location and following manufacturer's protocol (Roche 454 Life Sciences, Basel, Switzerland). Approximately 170,000 sequences were recovered. We used MSATCOMMANDER 0.8.2 to scan all sequences, detect the presence microsatellites (di and tetranucleotides repeats) and design primers when possible. Overall, 1302 putative microsatellites were found, 96 were selected and tested by PCR amplification on 8 individual samples in 10 µl reactions consisting of 5 µl master mix (Qiagen multiplex PCR kit), 1µl of primer mix (2µM), 0.5 µl of genomic DNA, and 3.5 µl of water. PCR thermal profile was performed according to manufacturer's protocol with annealing temperature of 60°C. Among these, 20 markers were successfully amplified, were polymorphic, and were subsequently amplified on 192

individuals with fluorescently labeled forward primers (either 6-FAM, PET, NED, or VIC).

Fragment analysis was conducted in an ABI 3730 XL genetic analyzer and allele sizes were scored using Genemapper 4.0 software (Applied Biosystems). A total of 11 markers were successfully amplified, yielded clear peaks that could be scored unambiguously, and amplified in all tested samples. Allelic frequencies, number of alleles, observed heterozygosities, and expected heterozygosities were estimated in Genalex v6.5 (Table 1). Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested in Genepop on the web v4.0 and corrected for multiple testing via False Discovery Rate estimation. None of the present 11 loci showed significant deviations from Hardy-Weinberg Equilibrium and no significant linkage disequilibrium was observed among any pair of loci.

## Acknowledgments

We would like to thank the Bioscience Core Lab of the King Abdullah University of Science and Technology (KAUST) for technical assistance. For general logistic and fieldwork assistance, we thank GP Jones, SR Thorrold, S Planes, HB Harrison, MA Priest, M Srinivasan, and N Tolou. Funding was provided in part by KAUST baseline research funds (to MLB), a KAUST Special Partnership Collaborative Fellowship (to MLB, SR Thorrold, and PS-A), and the Australian Research Council (ARC) (award FT0990835 to GR Almany) as well as ARC support to GP Jones.

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**Table 1. Characterization of 11 microsatellite loci for *Chaetodon vagabundus***

Locus	Primer sequence (5'-3')	Repeat motif	N <sub>a</sub>	Size range	H <sub>o</sub>	H <sub>e</sub>	Genebank acc. N.
<b>A114</b>	GCCCACAAAATCCTTTGAA TTGATGCAGGTCCACTGAAA	(CA) <sup>12</sup>	22	176-234	0.865	0.878	
<b>Cvag_08</b>	TGTGCCAGTTCAACACAC ACACACACACGTCTCCTCTG	(AC)(24)	8	116-138	0.219	0.229	
<b>C9</b>	GTATTGGCAACACCGTTGG GTAGCATGTCGGTGGTCTGA	(GACA) <sup>7</sup>	11	107-155	0.750	0.727	
<b>Cvag_32</b>	TCTCATAGCGCGAGTTCTC CCTTCACTGACATGCACACG	(AC) <sup>15</sup>	20	144-138	0.817	0.864	
<b>Cvag_96</b>	ATGCATCGCTGACAGGTTTG GTGAACACACCACTGAGCTG	(AC) <sup>12</sup>	26	269-333	0.916	0.908	
<b>Cvag_82</b>	AAAGGGACGCTGCTTGTTC ATCTTGGCTGGCTCTACGTG	(AC) <sup>12</sup>	15	142-190	0.771	0.767	
<b>A105</b>	CAGTGGAAACAAACAAC TTGC TGCTGGACAATATCCCACAG	GT <sup>16</sup>	21	156-222	0.911	0.908	
<b>Cvag55</b>	AAGGCTGCAGGTTGGTTTG AGCAGACTGTGACACTGAGG	(AC) <sup>15</sup>	13	146-176	0.536	0.510	
<b>Cvag77</b>	ACTGGTCATGTACAGAGGCC CTGGCTATCTGGGAGGTTGG	(AC) <sup>12</sup>	12	119-147	0.698	0.746	
<b>Cvag01</b>	ATACTCTCGCTGCTTCCCTG CGGCTGCACTTGACAGAAG	(AC) <sup>34</sup>	6	121-139	0.521	0.497	
<b>Cvag72</b>	ACTGAGAATGAGCCTGACCC TGTGTTGTGCCTGTTCATGC	(AC) <sup>12</sup>	13	101-129	0.661	0.729	

N<sub>a</sub> Number of observed alleles, H<sub>o</sub> observed heterozygosity, H<sub>e</sub> expected heterozygosity.

>A114 [organism=Chaetodon vagabundus]  
AGCCANCTTAAATNAAGATGANCTTCAGTGTGTTGAGAATGTTACATGACCCCCGACGCC  
CACAAAATCCTTTGAAACACAGTCTTGATTGAAAAAGAAAAAGAAAAATCCATGGT  
CACCCAGTAAAATAAAACAGACTCAGAAGTGCAGTGTGCCTACCTCTGCCCGCCAC  
ACACACACACACACACACACACACACACTCCTCTAAATATTAATGTGATTGATT  
CACTGGACCTGCATCAAATACACAGAATGACAGCCAGTCAGACTGATTAGCATACGAGC GCTTTGTTTTTT  
TGTTTTGTGCTCTCGGAGGGAAAAGACCCCTCGTTAACAGCCAGAAN >Cv08 [organism=Chaetodon  
vagabundus]  
GTCTACGGAGGGAGCAGGCAGCTCTGAGGTGAGAATGCAGAGTGGGAGGATCTCATTCC  
AATCCCCTGTGCCAGTTCAACACACACACACACACACACACACACACA  
CTAAACATCTGAGTAGTTAGGCCAGTCCACGCCAACATTAGAGGAGAGCAGCAGAGG  
AGACGTGTGTGTGCTATCTGCACTGAGAGTAAAAGCACAGTGACTACTTCTCTTT  
TGNGGTAGTGTGCGTGGCTCAGTGTGTGTGTGTGTGTGTGTGTGNGTGTGTG  
TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT  
>C9 [organism=Chaetodon vagabundus]  
NACCCAANCTCTGTATTGGCAACACCGTTGGTGGGACAGACAGACAGACAGACAGAC AGACAGGCCGTT  
ATCTAACGTAATATTACTGACTCTGTCACTTATAACATGTGACATG TCAGACCACCGACATGCTACAGCGT  
TGGCTCGTAAACAGTTACTGCTCGATTAAGTAC ACAATGGCAATAAACCGTCCCTAACATACAAAGCTA  
AGGGGCGAGGAGCAGACCCAT TGTAACCACTCACAATGATGTTCAATGAATCACAATTACGTTAAAGTTAA  
CAGCGATAAC GCAACTGTTACAACAGTTAGCCAGTTAGCTAACTCAGCATTCAACGCCAGTTAGCCGGC TA  
TTTGGCTGACATTATTTACAAGACATGGACGACAATTAAAACAAACGAGAAACGC TAACCAAAACACGT  
CACCTAACTTCCCGTAAACTTACGTTAGTTAGACGT AGCCAGCCCCGACANCCGNA  
>Cv32 [organism=Chaetodon vagabundus]  
ATTGGCAAACCATTCGCTGAATATGGAGGGAGAAAATATTTCTCATAGCGCGAGTTCC  
CATTGTGCGAGCAAGAGCGTGTGTGTGTGTGTGTGTGTGTGTGTGT  
TAGGTTGGAAGAAGTGGGTGCATCAGTAGGAAATCATCTGCTGCTGTCACTGCTCTAG  
CAGCACGTGTGCATGTCAGTGAAGGGAGGGATACGACGGAGAGAATGAGAGGAGAGTGTG  
AG  
>Cv96 [organism=Chaetodon vagabundus]  
TTGTTGAGCCTGAAGTGAACGCATGCTGACAGGTTGCTAAAGGCTTCCATCAA  
AGAGAAAGATTCCCTCATAATCTTGTCCGTCTGATCGCATGCCAAAACGGTAGCTCTGC  
TTTCTTACTTGTGACTGCAGACGAAATCTCAGCCCTTACTGTCTCCATATCTCTACT  
TTTCTTCACTGTCAGCATCATTTGCATACGTTGTAAAGGAAATGTGTGTGTGT  
GTGTGTGTGTGAGACAGACACAATGATGTCAGCTCAGTGGTGTGTTCAC  
>Cv82 [organism=Chaetodon vagabundus]  
TTGATGAAAGCCATTGGCTGGATTAGTTACACTCTCAATGGACCCGTGAGGTGAAGG  
GAGCCTCCGATCAGGATTAGTGAGTTAGTGGGCTGCAGACAGCCGGCGATGAAGAGG  
AAGAAGAACCTCTGTGTACTGGCTCCACTTCTCTCCAAAACAAAGGGACGCTGCTTGT  
CTGCAGACTAACGGAGGTGGGCTTCATGCTTGGCAGACTGACGACGTACGTGCGTGT  
GTTTGTATGTGTGTGTGTGTGTGTAAACGAGAGAAAGTGAAGCTGACAGTAG  
AGCCAGCCAAGATGCCAGTCTGAATG  
>A105 [organism=Chaetodon vagabundus]  
ACCCAAGCTTACATGTGAACAGACNGACAGACTATACAGTGGAAACAAACAACCTGCCTT GAAAGCAATAAG  
GGTATGTGTGCACATGTGTCAGTCTCGCTGTGTGTGTGTGTGT  
GTGTGTGTNGCGTGGTGTCTGTCTCAAAGCAATC TGGTTTAATGACTGTGGGATATTGTCCAGCATGTA  
ATTCANACAGGCAGGAATGTGTT GGATGTGTGTGTGTGTGTGTGT  
TATGTGTGAGTA GGTGCATTGGCAGTTGGCTGCATCCAGCCAGTCTTAGGAGTGTCCACGTCCAGTGGGG  
ACTGGCGTGTGATTGTAACTG >Cv55 [organism=Chaetodon vagabundus]  
GACTTGGTTAACGGCTGCAGGTTGGTTGACTCAGTCTGAGGAATGAATGTAAGTC  
ATGTAATGTCCCTGTGTGTGTGTGTGTGTGTGTGTGTGT  
CTCTGTGACGATGAAGATGAATCAGGATAGAAACGCCAGTGTGACAGTCTGCT >Cv77  
[organism=Chaetodon vagabundus]

