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New microsatellite DNA markers to resolve population structure of the convict surgeonfish, *Acanthurus triostegus*, and cross-species amplifications on thirteen other Acanthuridae

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4

5 **New microsatellite DNA markers to resolve population structure of the convict surgeonfish,**
6 ***Acanthurus triostegus*, and cross-species amplifications on thirteen other Acanthuridae**

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23 **Author identifying information**

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25 competing interest.

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27 numbers from MT876122-MT876135.

28 **Abstract**

29 Microsatellites are widely used to investigate connectivity and parentage in marine organisms. Despite
30 surgeonfish (Acanthuridae) being dominant members of most reef fish assemblages and having an
31 ecological key role in coral reef ecosystems, there is limited information describing the scale at which
32 populations are connected and very few microsatellite markers have been screened. Here, we
33 developed fourteen microsatellite markers for the convict surgeonfish *Acanthurus triostegus* with the
34 aim to infer its genetic connectivity throughout its distribution range. Genetic diversity and variability
35 was tested over 152 fishes sampled from four locations across the Indo-Pacific: Mayotte (Western
36 Indian Ocean), Papua New Guinea and New Caledonia (Southwestern Pacific Ocean), and Moorea
37 (French Polynesia). Over all locations, the number of alleles per locus varied from 5 to 24 per locus,
38 and expected heterozygosities ranged from 0.468 to 0.941. Significant deviations from Hardy-
39 Weinberg equilibrium were detected for two loci in two to three locations and were attributed to the
40 presence of null alleles. These markers revealed for the first time a strong and significant
41 distinctiveness between Indian Ocean and Pacific Ocean *A. triostegus* populations. We further
42 conducted cross-species amplification tests in thirteen Pacific congener species to investigate the
43 possible use of these microsatellites in other Acanthuridae species. The phylogenetic placement of *A.*
44 *triostegus* branching off from the clade containing nearly all *Acanthurus* + *Ctenochaetus* species likely
45 explain the rather good transferability of these microsatellite markers towards other Acanthuridae
46 species. This suggests that this fourteen new microsatellite loci will be helpful tools not only for
47 inferring population structure of various surgeonfish but also to clarify systematic relationships among
48 Acanthuridae.

49

50 **Keywords**

51 Coral reef fish; microsatellites; connectivity; Indo-Pacific; genetic structure; surgeonfish

52

53

54

55 **Introduction**

56 Acanthuridae (surgeonfishes, tangs and unicornfishes) are dominant fish taxa in most coral reefs, with
57 an ecological key role in preventing shifts from coral- to algal-dominance following disturbance [1].

58 Yet, Acanthuridae are under increasing pressure: they are heavily targeted by artisanal fishing (several
59 unicornfish are highly prized in tropical Indo-Pacific fisheries) and/or as ornamental species, being in
60 the top 10 of the most-frequently exported aquarium fish in trade [2–4]. Despite its importance above,
61 and although it is one of the most widespread coral reef fish family in coral reefs, it is one of the least
62 studied in terms of population genetic structure. Only a few studies have investigated
63 phylogeographical patterns and/or population genetic connectivity in these fishes, and for the majority,
64 the genetic variation was inferred using mitochondrial DNA (mtDNA) markers [5–13]. Only a limited
65 number of studies used nuclear microsatellite markers, despite their high resolving power for detecting
66 divergence [14, 15].

67 Currently available Acanthuridae microsatellite makers were designed from only 3 out of the 84
68 species of Acanthuridae: one tang, one unicornfish and one surgeonfish. The genetic structure of the
69 yellow tang *Zebrasoma flavescens* was investigated throughout its Pacific distribution range using 23
70 specific microsatellites loci [16]. Compared to previously used mtDNA markers, microsatellite
71 markers provided finer estimates of the spatial subdivision of the Hawaiian population [17] and
72 allowed to infer small scale larval dispersal through genetic parentage analyses in yellow tang off the
73 Island of Hawai'i [18]. In the unicornfish *Naso unicornis*, the genetic relatedness among recruits was
74 inferred using 15 specially developed microsatellites loci [19], revealing a broad-scale genetic
75 connectivity across the southern Marianas Islands [20]. Lastly, ten microsatellite markers developed
76 from hybrids of *Acanthurus nigricans* x *A. leucosternon* [21] were specifically used to study
77 introgression patterns among four species of Acanthurids and investigate evolutionary processes
78 leading to hybridization among closely related species [22]. These microsatellites were latter used in
79 two related studies exploring the genetic structure and connectivity of two species of Acanthurids at
80 the Eastern African region scale, *A. leucosternon* and *A. triostegus*, revealing homogeneous panmictic
81 populations at this spatial scale for the two species [23, 24].

82 The convict surgeonfish *A. triostegus* is found throughout the tropical Indo-Pacific, from South
83 African to Baja Californian reefs. The genetic population structure of *A. triostegus* across its entire
84 range, using either allozymes or mtDNA sequences [13, 25] revealed globally congruent results: 1) a
85 marked genetic differentiation of populations from the Hawaiian archipelago, suggesting
86 biogeographic vicariance as an evolutionary process leading to the differentiation of the *A. triostegus*
87 populations in this archipelago; and 2) a significant correlation between genetic differentiation and
88 geographic distance among the remaining populations, indicative of an isolation by distance.
89 Significant genetic differentiation between the Indian and Pacific Ocean populations was found,
90 though only 8.3% of all pairwise comparisons were significant [13]. In addition, no significant
91 differentiation was found across the East Pacific Barrier [6], suggesting a great dispersal potential of
92 this species. Nevertheless, one discrepancy among nuclear and mitochondrial markers remains with
93 the Marquesas population being as much differentiated than the Hawaiian archipelago from the rest of
94 the Pacific populations based on allozymes [25] but not based on mtDNA sequences [6, 12, 13]. This
95 incongruity calls for additional type of markers to be used.
96 Here we report the development of fourteen microsatellite markers whose power to detect genetic
97 subdivision are tested across four populations sampled across the distribution range of the species. In
98 addition we tested cross-amplification on thirteen congeners to investigate their potential to be used
99 more widely within the Acanthuridae family.

100

101 **Material and Methods**

102 ***Microsatellite library development and primer selection***

103 Approximately 20 ng of genomic DNA was isolated from muscle tissue of one *A. triostegus* sampled
104 in Moorea, French Polynesia and preserved in 80% EtOH. Size-selected fragments from genomic
105 DNA were enriched for SSR content by using magnetic streptavidin beads and biotin-labeled CT and
106 GT repeat oligonucleotides. The SSR-enriched library was analyzed on a Roche 454 platform using
107 the GS FLX Titanium reagents. A total of 21'986 reads had an average length of 128 base pairs. Of
108 these, 3'482 contained a microsatellite insert with a tetra- or a trinucleotide of at least 6 repeat units or
109 a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 1'042 reads and 32

110 loci were tested for PCR amplification and polymorphism on 8 individuals sampled in Moorea using
111 the method fully detailed in Schuelke [26]. Genomic DNA was isolated from fin clips using Gentra
112 Puregene Tissue Kit (Qiagen). Forward primers were labelled with a fluorochrome (6-FAM) by
113 adding a universal 18-bp M13 tail at their 5'-end (5'-TGTAACGACGGCCAGT-3'). PCRs were
114 performed in a total volume of 10 μ L with 1X Qiagen buffer stock, 0.04 μ M of forward primer tagged
115 with the M13 tail, 0.16 μ M of reverse primer, 0.16 μ M of fluorescent dyed M13 primer, 0.5 U of
116 Hotstar Taq and 10 ng of genomic DNA. The following thermocycling program was used: 95°C for 15
117 min + 30 \times (95°C for 30 s, 56°C for 45 s, 72°C for 45 s) + 8 \times (95°C for 30 s, 53°C for 45 s, 72°C for
118 45 s) + 72°C for 30 min. PCR products were genotyped using an ABI3730 sequencer (Applied
119 Biosystems) with the GS-LIZ-500 Size Standard (Applied Biosystems). Microsatellite peaks in the
120 electropherograms were examined and the most promising 14 microsatellite loci were selected for
121 further genotyping and analysis based on the fact that (1) primer pairs amplified fragments in all eight
122 individuals, (2) the number of different alleles was higher than 25% (i.e. at least 4 out of the 16
123 possible amplified alleles), and (3) they did not amplify multiple fragments (Table 1).

124

125 ***Polymorphism and cross - amplification testing***

126 The final set of 14 microsatellite loci selected were further characterized by genotyping individuals
127 sampled in four locations: three islands of the Pacific Ocean—Moorea in French Polynesia, Grande
128 Terre in New Caledonia, and Loloata Island in Papua New Guinea and one island in the Western
129 Indian Ocean, Mayotte (Table 1). Genomic DNA was isolated from fin clips using Gentra Puregene
130 Tissue Kit (Qiagen). PCR reactions were performed using Type-It Microsatellite (Qiagen) in two
131 distinct multiplexes of 5 μ l final volume containing 1X Master Mix, 0.5X of Q-solution, 0.1 μ M of
132 each primer (fluorescent-labeled forward primer 6-FAM, PET, NED or VIC) and 50 to 150 ng of
133 DNA template (Table 1). All PCRs were conducted in GeneAMP PCR System 9700 (Applied
134 Biosystems) and a unique program was used to amplify the two multiplexes, consisting of 5 min at
135 94°C, 28 cycles at 95°C for 30 s, 58°C for 90 s and 72°C for 30 s, and a final step at 60°C for 30 min.
136 Fluorescent PCR fragments were visualized on an ABI 3130XL Genetic Analyser (Applied

137 Biosystems) with GS-500-LIZ (Applied Biosystems). Alleles were sized using GeneMapper®
138 (Applied Biosystems).
139 Cross-species amplification was tested on 13 species of 3 genus of Acanthuridae sampled in New
140 Caledonia (*A. albipectoralis*, *A. blochii*, *A. dussumieri*, *A. nigricauda*, *A. nigrofuscus*, *A. olivaceus*) and
141 in Moorea (*A. nigricans*, *A. pyroferus*, *A. xantheptorus*, *Ctenochaetus birotatus*, *C. flavicauda*, *C.*
142 *striatus* and *Naso lituratus*). PCR were conducted in the exact same conditions as described above. We
143 will then only report the number of loci that amplified, the number of alleles observed for each loci
144 and the size range of the alleles.

145

146 ***Data analysis for Acanthurus triostegus***

147 Genetic diversity within samples was estimated at the fourteen loci from the observed (H_O) and
148 expected (H_E) heterozygosities in GENETIX 4.05 [27]. Deviations from Hardy–Weinberg (HW)
149 equilibrium were estimated for each loci using Weir & Cockerham’s [28] estimator of the F_{IS}
150 inbreeding coefficient, and departures from HW expectations were tested using the probability test in
151 GENEPOP v 4.7.5 on the web [29, 30] with default Markov chain parameters and applying a standard
152 Bonferroni correction [31]. Genotypic linkage disequilibrium among loci was tested using GENEPOP
153 for each sample. MICRO-CHECKER 2.2.3 [32] was used to screen for the presence of null alleles,
154 scoring error due to stuttering and large allele dropout.

155 Pairwise genetic divergence between samples was estimated using Weir & Cockerham’s [27]
156 multilocus estimator of F_{ST} ($\hat{\theta}$) in GENETIX. The genic differentiation for each population pair was
157 tested using the exact G test of GENEPOP. The sequential Bonferroni correction [31] was applied for
158 each test. The population structure was further examined using a Discriminant Analysis of Principal
159 Components (DAPC) procedure described by Jombart et al. [33] to identify the number of genetically
160 distinct clusters (K) present in the dataset. We chose this method as it does not make any assumption
161 about HWE or linkage equilibrium and transforms genotypes using PCA as a prior step to a
162 discriminant analysis. DAPC was run using the *adegenet* package [34] for R (R Development Core
163 Team 2016). For comparison, we also performed a Bayesian analysis using STRUCTURE 2.3.4 [35]
164 to determine the most likely number of genetically distinct clusters (K) among the 156 genotyped

165 individuals. Conditions were set to 500 000 chain length after a burn-in of 50 000, assuming admixture
166 and using the location prior option. Percentage of membership of each individual to each cluster ($K=1$
167 to $K=5$) were obtained pooling the results of 10 independent runs with CLUMPP 1.1.2 [36] and were
168 graphically displayed using DISTRUCT [37].

169

170 **Results and discussion**

171 *Characterization of microsatellite loci*

172 Sequences of the 14 selected loci are available on GenBank with accession numbers from MT876122-
173 MT876135 and primer sequences are presented in Table 1. Within the sample from Moorea (the
174 original location of the fish used to isolate all the microsatellite loci), between 5 and 22 alleles (mean
175 = 14.14) were observed per locus, very similar to what found in *Naso unicornis* [19] and *Zebrasoma*
176 *flavescens* [16] microsatellite loci. Expected heterozygosity values ranged from 0.692 to 0.925 (0.840
177 over all loci) (Table 1). No significant genotypic linkage disequilibrium among loci was found but
178 significant deviation from HW expectations was observed in a single locus, Acatri_13915 ($F_{IS}=0.523$,
179 $P < 0.0001$). This excess of homozygotes was attributed to the presence of null alleles. For
180 Acatri_09917, null alleles were also found to be present, but with no significant departure from HW
181 expectations.

182 Within the three other samples (New Caledonia, Loloata Isl. and Mayotte), the genetic diversity was
183 similar, with a number of alleles per locus between 7 and 24 and mean number of alleles ranging from
184 11.21 in New Caledonia to 15.07 in Mayotte (Table 1). Expected heterozygosity values ranged from
185 0.468 to 0.941 (with mean values over all loci ranging from 0.822 in Mayotte to 0.850 in Loloata Isl.).
186 Significant deviations from HW expectations were observed for Acatri_13915 ($F_{IS}=0.237$, $P < 0.0001$)
187 in Mayotte, and for Acatri_09917 in Mayotte ($F_{IS}=0.173$, $P < 0.0001$), Loloata Isl. ($F_{IS}=0.210$, P
188 < 0.0001) and New Caledonia ($F_{IS}=0.382$, $P < 0.0001$). In all these cases, the deficits of homozygotes
189 were attributed to the occurrence of null alleles.

190

191 *Detection of significant genetic structure*

192 Pairwise genetic divergence among samples were significant for all pairwise comparisons except
193 between New Caledonia and Loloata island, the closest sampled populations. Genetic differentiation
194 estimates ranged from 0.00097 (exact P -value=0.087) between New Caledonia and Loloata island to
195 0.03786 ($P < 10^{-11}$) between Mayotte and Loloata Isl.

196 The strong distinctiveness of the Indian Ocean sample, Mayotte, was further confirmed by the two
197 different clustering approaches. The STRUCTURE analysis revealed that the best partition was
198 obtained for $K=2$, with all Indian Ocean individuals (i.e. Mayotte) belonging to cluster 1, and all
199 Pacific Ocean individuals (i.e. Loloata Isl., New Caledonia and Moorea) belonging to cluster 2 (Fig.
200 1a). The results of DAPC (Fig. 1b and c) are largely consistent with those of the Bayesian analysis
201 showing that all analysed individuals are separated in two distinct genetic clusters, one cluster being
202 represented by most of the Indian Ocean individuals, and the second, by most of the Pacific Ocean
203 individuals. The occurrence of Pacific Ocean individuals belonging to the Indian Ocean cluster (Fig.
204 1c) may represent directional gene flow from the Pacific Ocean to the Indian Ocean and/or homoplasy.

205

206 ***Cross-species amplifications***

207 Cross-species amplification tests (Table 2) resulted in 10 loci amplifying in *A. nigricans* (*Anig*), 8 loci
208 amplifying in *A. pyroferus* (*Apyr*), *C. striatus* (*Cstri*) and *C. birotatus* (*Cbir*), 7 loci amplifying in
209 *A. xanteptorus* (*Axan*) and *A. olivaceus* (*Aoli*), 6 loci amplifying in *A. albipectoralis* (*Aalb*),
210 *A. nigricauda* (*Anic*), *A. nigrofuscus* (*Anif*) and *Ctenochaetus flavicauda* (*Cfla*), 5 loci amplifying in
211 *A. dussmieri* (*Adus*), and 4 loci amplifying in *Naso lituratus* (*Nlit*) and *A. blochii* (*Ablo*), though lower
212 annealing temperatures may be tested to improve these successes. The rather good transferability of
213 *A. triostegus* microsatellite markers towards other Acanthuridae species considered here may be
214 attributed to the phylogenetic placement of *A. triostegus* branching off from the clade containing all
215 *Acanthurus* + *Ctenochaetus* species but *A. thompsoni* [38].

216 These markers are currently being - or will be - used to investigate historical biogeography, population
217 connectivity at various spatial scales, larval recruitment patterns, hybridization, and speciation in reef
218 fishes.

219

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223

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227

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230 a research permit provided by the Province Sud of New Caledonia (#3959-2011/ARR/DENV).

231

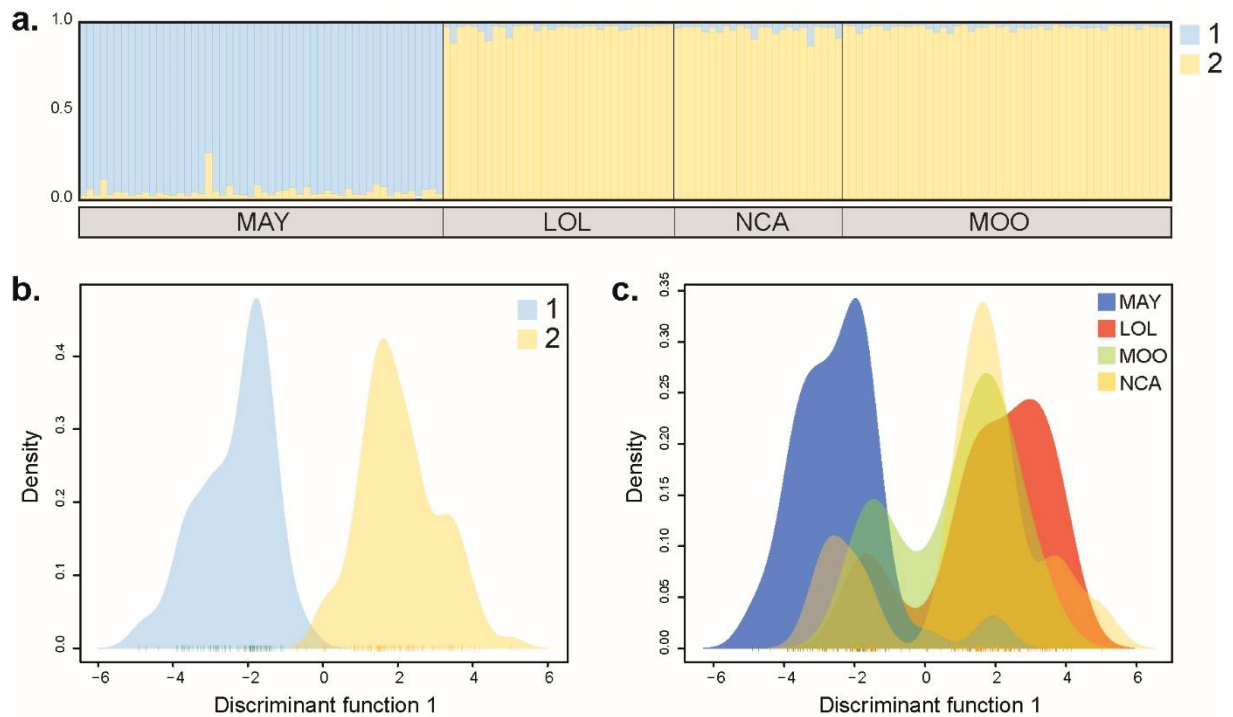
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334 **Figure 1:** Population differentiation of *A. triostegus* populations based on the analysis of 14
 335 microsatellite loci. STRUCTURE assignment plot showing individual's posterior probabilities of
 336 membership to each of the two clusters (a). DAPC scatter plots of the first discriminant function of the
 337 Principal Component Analysis (PCA) representing individual densities based on their membership to
 338 each cluster (b), or based on their population of origin (c). MAY: Mayotte, south Western Indian
 339 Ocean; LOL: Loloata Island, Papua New Guinea; NCA: New Caledonia and MOO: Moorea, French
 340 Polynesia.

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346 **Table 1:** Characteristics of 14 microsatellite loci isolated in *Acanthurus triostegus*. *Dye* = fluorescent dye used for each forward primer, *Mix* = multiplex in which each loci
347 was amplified. *Size*: observed amplified fragment size range (in bp). Genetic diversity indexes per loci and over all loci, within each sample (MOO: Moorea; NCA: New
348 Caledonia; LOL: Loloata Island; MAY: Mayotte). *N*= Number of analyzed individuals; *Na*: Number of alleles; *H_E*: expected heterozygosity; *H_O*: observed heterozygosity; *F_{IS}*:
349 Weir & Cockerham's (1984) inbreeding coefficient. * = significant after standard Bonferroni correction.

	Primer sequence 5'-3'	Repeat array	Dye (Mix)	Size	MOO (N=47)			NCA (N=24)			LOL (N=33)			MAY (N=52)		
					Na	H _E /H _O	F _{IS}	Na	H _E /H _O	F _{IS}	Na	H _E /H _O	F _{IS}	Na	H _E /H _O	F _{IS}
<i>Acatri_03083</i>	F: CATTGAGTCACCGCATCCTG R: GCTGAGTTCAGAGCATTGGC	(AC) ₁₃	VIC (2)	161-199	15	0.826	0.032	10	0.827	0.114	8	0.828	0.173	12	0.770	0.011
<i>Acatri_04614</i>	F: TCAGTGCTGCTGTGAATTGG R: CTCATGCACAAACACAAGAC	(TG) ₁₄	VIC (1)	134-160	13	0.866	-0.043	12	0.880	-0.015	10	0.846	0.012	12	0.843	-0.058
<i>Acatri_05455</i>	F: ATACGGACACACAAGTGGGC R: AGTTTAATTGGTGGCGATGAC	(CA) ₁₄	PET (2)	83-161	22	0.921	0.022	16	0.920	0.062	18	0.905	0.011	14	0.760	-0.025
<i>Acatri_09735</i>	F: TGTCTATTGTTTTGGACAAGGAGC R: TGGTCCAACCTGAGACAGC	(GT) ₁₈	NED (2)	98-140	21	0.910	0.031	16	0.913	0.077	20	0.912	0.085	24	0.906	0.140
<i>Acatri_09917</i>	F: GTGCTCTCAAAGACACAGCC R: CATGCCCCATTTCGACAAAAC	(TCTG) ₁₈	NED (2)	190-302	20	0.925	0.137	17	0.927	0.382*	21	0.941	0.210*	20	0.932	0.173*
<i>Acatri_10969</i>	F: GGAGCAAATACGAGCGAGTG R: AAGGACGTAGTCAGCACACC	(TG) ₁₅	6-FAM (2)	196-218	10	0.806	-0.098	12	0.870	0.159	12	0.876	0.012	15	0.874	0.042
<i>Acatri_13144</i>	F: TCTGTTTAAATGCACAAACGC R: GTGTGTCTCCAGATCCAGGC	(CA) ₁₅	6-FAM (1)	134-142	5	0.692	0.175	7	0.744	0.125	7	0.757	0.015	10	0.769	0.041
<i>Acatri_13915</i>	F: CAGTCTGCTGAACCTCCTCC R: TCGAATCAATCTGTGCGTGC	(AC) ₁₃	PET (1)	90-130	13	0.829	0.523*	10	0.716	0.103	13	0.863	0.045	22	0.919	0.237*
<i>Acatri_14579</i>	F: ACACCAGCACGTCTAGGAAG R: ACTGCTGGATAACAGTGTGTG	(CA) ₁₄	VIC (1)	86-120	13	0.757	0.092	10	0.804	-0.067	12	0.793	0.060	11	0.468	0.065
<i>Acatri_15132</i>	F: GAGCTTGACCTACATGTGCC R: ATCACTTCTCCTGCGTGGAC	(TG) ₁₆	NED (1)	86-124	11	0.791	-0.028	8	0.728	0.276	8	0.752	0.088	11	0.745	0.080
<i>Acatri_15723</i>	F: GGCTAGCTGAGCACATTTCAG R: AGCATCGTAGGTATGCGGAG	(GT) ₁₃	6-FAM (1)	84-104	10	0.838	0.083	8	0.823	0.059	8	0.828	0.064	7	0.824	0.053
<i>Acatri_16496</i>	F: ATCCTCTGACAATAGGCCCG R: TGCAGACACTATGTAGTCCACC	(GT) ₁₂	PET (1)	146-170	10	0.817	0.066	8	0.817	-0.089	11	0.820	-0.020	14	0.867	0.012
<i>Acatri_17233</i>	F: GGGCTCGTTTATCTGCAAGG R: GTAAGTGATCTCGGTTAGATGC	(GT) ₁₃	NED (1)	126-166	17	0.912	-0.006	11	0.891	0.183	16	0.888	0.025	17	0.908	-0.068
<i>Acatri_18344</i>	F: TCAGCCAGCCGAATCTGAAC R: CTCACCAAGCCATGTTAGCC	(TG) ₁₉	6-FAM (2)	106-142	18	0.863	0.074	13	0.872	0.160	17	0.893	0.167	22	0.921	0.008
<i>over all loci</i>					14.14	0.840		11.21	0.838		12.93	0.850		15.07	0.822	
						0.787			0.765			0.805			0.787	

Table 2: Cross species amplification of fourteen loci isolated in *Acanthurus triostegus* tested in thirteen Acanthuridae species. For each species, the total number of individual used is indicated in parenthesis (n=). Then for each locus, the first number indicates the number of amplified alleles, followed by the number of amplified individuals in parenthesis. Range: range size of the amplified fragments (in base pairs). - : no amplification.

	<i>Acatri_03083</i>	<i>Acatri_04614</i>	<i>Acatri_05455</i>	<i>Acatri_09735</i>	<i>Acatri_09917</i>	<i>Acatri_10969</i>	<i>Acatri_13144</i>	<i>Acatri_13915</i>	<i>Acatri_14579</i>	<i>Acatri_15132</i>	<i>Acatri_15723</i>	<i>Acatri_16496</i>	<i>Acatri_17233</i>	<i>Acatri_18344</i>
<i>Aalb</i> (n=8)	3 (8)	-	2 (5)	5 (8)	-	-	7 (7)	-	3 (7)	-	-	-	-	6 (8)
Range	173-191	-	105-107	104-136	-	-	164-212	-	78-84	-	-	-	-	108-124
<i>Ablo</i> (n=5)	5 (5)	-	-	5 (4)	-	-	-	-	4 (3)	-	-	-	-	3 (5)
Range	177-187	-	-	90-122	-	-	-	-	90-100	-	-	-	-	104-126
<i>Adus</i> (n=3)	3 (3)	-	4 (2)	2 (3)	-	-	-	-	2 (3)	-	-	-	-	3 (3)
Range	177-189	-	93-111	104-106	-	-	-	-	80-82	-	-	-	-	116-120
<i>Anic</i> (n=1)	1 (1)	-	1 (1)	1 (1)	-	-	1 (1)	-	-	-	1 (1)	-	-	1 (1)
Range	175	-	99	104	-	-	150	-	-	-	85	-	-	110
<i>Anif</i> (n=4)	4 (4)	-	1 (2)	6 (4)	-	-	-	-	-	3 (4)	2 (4)	-	-	6 (4)
Range	171-203	-	117	92-124	-	-	-	-	-	88-96	86-88	-	-	94-128
<i>Anig</i> (n=8)	3 (8)	4 (8)	9 (7)	9 (7)	-	3 (6)	7 (8)	-	-	4 (8)	-	4 (8)	2 (8)	6 (7)
Range	173-177	185-195	99-161	90-118	-	194-198	162-184	-	-	96-114	-	138-140	128-148	80-94
<i>Aoli</i> (n=3)	2 (3)	-	2 (3)	3 (3)	-	-	1 (1)	-	1 (1)	-	2 (1)	-	-	3 (3)
Range	169-177	-	101-103	94-98	-	-	148	-	80	-	86-96	-	-	106-124
<i>Apyr</i> (n=8)	6 (8)	-	4 (8)	4 (8)	-	-	6 (7)	-	2 (5)	6 (8)	4 (6)	-	-	3 (8)
Range	173-187	-	95-101	88-94	-	-	146-168	-	78-88	84-98	88-94	-	-	80-86
<i>Axan</i> (n=8)	5 (8)	1 (4)	-	1 (6)	-	-	2 (3)	-	5 (6)	-	2 (5)	-	-	4 (7)
Range	177-185	176	-	102	-	-	140-162	-	84-94	-	84-86	-	-	110-120
<i>Cbir</i> (n=1)	2 (1)	-	2 (1)	2 (1)	-	-	1 (1)	-	-	1 (1)	2 (1)	-	1 (1)	1 (1)
Range	169-179	-	97-99	106-110	-	-	144	-	-	102	84-92	-	132	98
<i>Ctfla</i> (n=6)	4 (6)	5 (6)	3 (6)	6 (6)	-	-	-	-	-	6 (6)	-	-	-	6 (6)
Range	165-179	130-156	93-99	92-132	-	-	-	-	-	80-98	-	-	-	88-102
<i>Cstr</i> (n=8)	10 (8)	1 (1)	-	9 (8)	-	2 (3)	7 (6)	-	6 (5)	4 (6)	-	-	-	4 (8)
Range	165-223	138	-	94-120	-	216-220	116-206	-	90-114	94-104	-	-	-	86-94
<i>Nlit</i> (n=8)	3 (8)	-	-	-	-	-	-	-	-	2 (4)	-	-	4 (7)	3 (8)
Range	175-187	-	-	-	-	-	-	-	-	94-110	-	-	124-140	96-122

Aalb: Acanthurus albipectoralis; Ablo : Acanthurus blochii; Adus: Acanthurus dussumieri; Anic: Acanthurus nigricauda; Anif: Acanthurus nigrofuscus; Anig : Acanthurus nigricans; Aoli: Acanthurus olivaceus; Apyr : Acanthurus pyroferus; Axan : Acanthurus xanteporus; Cbir : Ctenochaetus birotatus; Ctfla : Ctenochaetus flavicauda; Cstr : Ctenochaetus striatus; Nlit : Naso lituratus.