

**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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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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22

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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

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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  $\mu$ L with 1X Qiagen buffer stock, 0.04  $\mu$ M of forward primer tagged  
115 with the M13 tail, 0.16  $\mu$ M of reverse primer, 0.16  $\mu$ 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  $\mu$ l final volume containing 1X Master Mix, 0.5X of Q-solution, 0.1  $\mu$ 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. dussmieri*, *A. nigricauda*, *A. nigrofuscus*, *A. olivaceus*) and  
141 in Moorea (*A. nigricans*, *A. pyroferus*, *A. xanteptorus*, *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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231

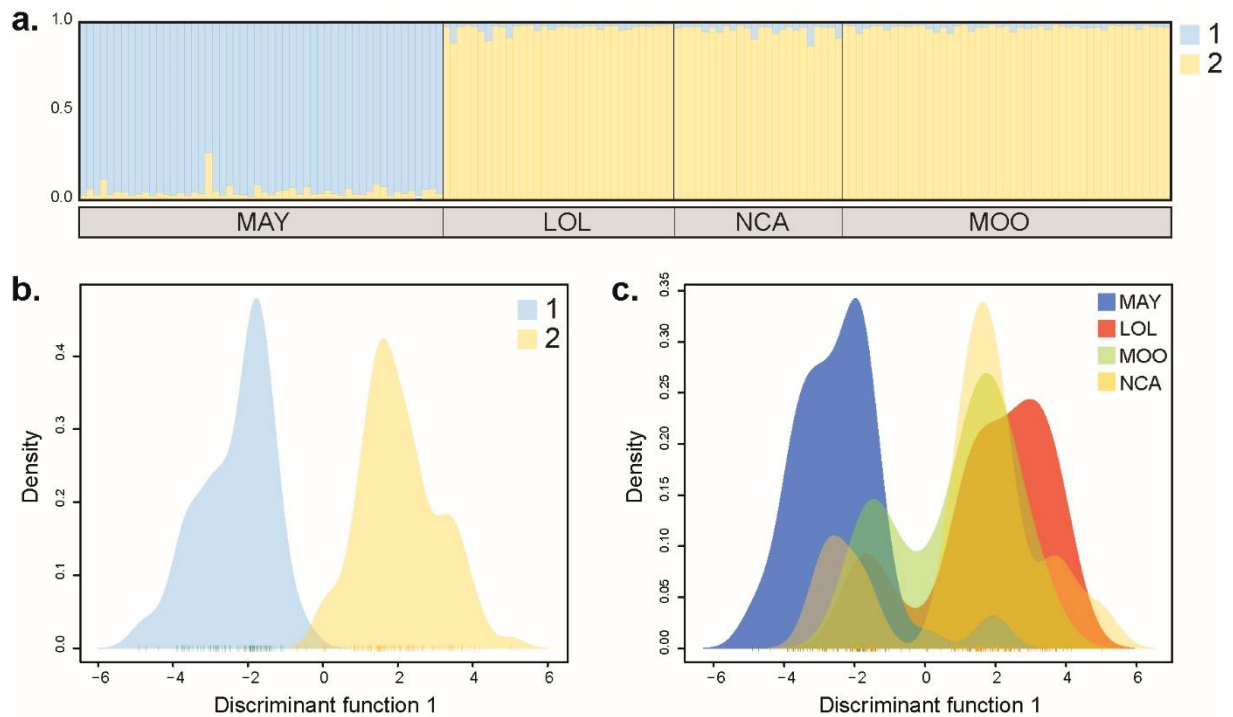
## 232 **References**

- 233 1. Marshall A, Mumby PJ (2015) The role of surgeonfish (Acanthuridae) in maintaining algal turf biomass  
234 on coral reefs. *J Exp Mar Biol Ecol* 473:152–160. <https://doi.org/10.1016/j.jembe.2015.09.002>
- 235 2. Wabnitz C, Taylors M, Green E, Razak T (2003) From ocean to aquarium: the global trade in marine  
236 ornamental species. UNEP World Conservation Monitoring Centre, Cambridge, UK
- 237 3. Rhyne AL, Tlusty MF, Schofield PJ, et al (2012) Revealing the appetite of the marine aquarium fish trade:  
238 the volume and biodiversity of fish imported into the United States. *PLOS ONE* 7:e35808.  
239 <https://doi.org/10.1371/journal.pone.0035808>
- 240 4. Biondo MV (2017) Quantifying the trade in marine ornamental fishes into Switzerland and an estimation  
241 of imports from the European Union. *Global Ecology and Conservation* 11:95–105.  
242 <https://doi.org/10.1016/j.gecco.2017.05.006>
- 243 5. Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the  
244 comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). *Mol Ecol* 11:243–  
245 251. <https://doi.org/10.1046/j.0962-1083.2001.01431.x>
- 246 6. Lessios HA, Robertson DR (2006) Crossing the impassable: genetic connections in 20 reef fishes across  
247 the eastern Pacific barrier. *Proc Biol Sci* 273:2201–8. <https://doi.org/10.1098/rspb.2006.3543>
- 248 7. Horne JB, van Herwerden L, Choat JH, Robertson DR (2008) High population connectivity across the  
249 Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners. *Mol Phyl Evol*  
250 49:629–638. <https://doi.org/10.1016/j.ympev.2008.08.023>
- 251 8. Eble JA, Toonen RJ, Bowen BW (2009) Endemism and dispersal: comparative phylogeography of three  
252 surgeonfishes across the Hawaiian Archipelago. *Mar Biol* 156:689–698. <https://doi.org/10.1007/s00227-008-1119-4>  
253

- 254 9. DiBattista JD, Wilcox C, Craig MT, et al (2011) Phylogeography of the Pacific blueline surgeonfish,  
255 *Acanthurus nigroris*, reveals high genetic connectivity and a cryptic endemic species in the Hawaiian  
256 archipelago. *J Mar Biol* 2011:1–17. <https://doi.org/10.1155/2011/839134>
- 257 10. DiBattista JD, Berumen ML, Gaither MR, et al (2013) After continents divide: comparative  
258 phylogeography of reef fishes from the Red Sea and Indian Ocean. *J Biogeog* 40:1170–1181.  
259 <https://doi.org/10.1111/jbi.12068>
- 260 11. Selkoe KA, Gaggiotti OE, Bowen BW, Toonen RJ (2014) Emergent patterns of population genetic  
261 structure for a coral reef community. *Mol Ecol* 23:3064–3079. <https://doi.org/10.1111/mec.12804>
- 262 12. Liggins L, Trembl EA, Possingham HP, Riginos C (2016) Seascape features, rather than dispersal traits,  
263 predict spatial genetic patterns in co-distributed reef fishes. *J Biogeog* 43:256–267.  
264 <https://doi.org/10.1111/jbi.12647>
- 265 13. Otwoma LM, Diemel V, Reuter H, et al (2018) Genetic population structure of the convict surgeonfish  
266 *Acanthurus triostegus*: a phylogeographic reassessment across its range. *J Fish Biol* 93:597–608.  
267 <https://doi.org/10.1111/jfb.13686>
- 268 14. Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating  
269 microsatellite markers. *Ecol Lett* 9:615–29. <https://doi.org/10.1111/j.1461-0248.2006.00889.x>
- 270 15. Ryman N, Palm S, André C, et al (2006) Power for detecting genetic divergence: differences between  
271 statistical methods and marker loci. *Mol Ecol* 15:2031–2045. <https://doi.org/10.1111/j.1365->  
272 [294X.2006.02839.x](https://doi.org/10.1111/j.1365-294X.2006.02839.x)
- 273 16. Christie MR, Eble JA (2009) Isolation and characterization of 23 microsatellite loci in the yellow tang,  
274 *Zebrasoma flavescens* (Pisces: Acanthuridae). *Mol Ecol Resour* 9:544–6. <https://doi.org/10.1111/j.1755->  
275 [0998.2008.02354.x](https://doi.org/10.1111/j.1755-0998.2008.02354.x)
- 276 17. Eble J, Toonen R, Sorenson L, et al (2011) Escaping paradise: larval export from Hawaii in an Indo-  
277 Pacific reef fish, the yellow tang *Zebrasoma flavescens*. *Mar Ecol Prog Ser* 428:245–258.  
278 <https://doi.org/10.3354/meps09083>
- 279 18. Christie MR, Tissot BN, Albins MA, et al (2010) Larval connectivity in an effective network of marine  
280 protected areas. *PLoS One* 5:e15715. <https://doi.org/10.1371/journal.pone.0015715>
- 281 19. Horne JB, McIlwain JL, Herwerden L van (2010) Isolation of 15 new polymorphic microsatellite markers  
282 from the blue-spine unicornfish *Naso unicornis*. *Conserv Genet Resour* 2:191–194.  
283 <https://doi.org/10.1007/s12686-009-9129-1>
- 284 20. Horne JB, van Herwerden L, Abellana S, McIlwain JL (2013) Observations of migrant exchange and  
285 mixing in a coral reef fish metapopulation link scales of marine population connectivity. *J Hered* 104:532–  
286 546. <https://doi.org/10.1093/jhered/est021>
- 287 21. DiBattista JD, Feldheim KA, Bowen BW (2011) Microsatellite DNA markers to resolve population  
288 structure and hybridization of two closely related surgeonfish species, *Acanthurus nigricans* and  
289 *Acanthurus leucosternon*. *Conserv Genet Resour* 3:159–162. <https://doi.org/10.1007/s12686-010-9313-3>
- 290 22. DiBattista JD, Whitney J, Craig MT, et al (2016) Surgeons and suture zones: Hybridization among four  
291 surgeonfish species in the Indo-Pacific with variable evolutionary outcomes. *Mol Phyl Evol* 101:203–215.  
292 <https://doi.org/10.1016/j.ympev.2016.04.036>
- 293 23. Otwoma LM, Reuter H (2019) Do differences in mating behaviour lead to differences in connectivity  
294 patterns of reef fishes? Insights from two sympatric surgeonfish species in the Indian Ocean. *Mar Environ*  
295 *Res* 151:104760. <https://doi.org/10.1016/j.marenvres.2019.104760>
- 296 24. Otwoma LM, Reuter H, Timm J, Meyer A (2018) Genetic connectivity in a herbivorous coral reef fish  
297 (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African region. *Hydrobiologia* 806:237–250.  
298 <https://doi.org/10.1007/s10750-017-3363-4>

- 299 25. Planes S, Fauvelot C (2002) Isolation by distance and vicariance drive genetic structure of a coral reef fish  
300 in the Pacific Ocean. *Evolution* 56:378–399
- 301 26. Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nat Biotechnol*  
302 18:233–234. <https://doi.org/10.1038/72708>
- 303 27. Belkhir K, Borsa P, Chikhi L, et al (1996) GENETIX 4.05, logiciel sous Windows TM pour la génétique  
304 des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de  
305 Montpellier II, Montpellier (France).
- 306 28. Weir BS, Cockerham CC (1984) Estimating F-Statistics for the analysis of population structure. *Evolution*  
307 38:1358–1370. <https://doi.org/10.2307/2408641>
- 308 29. Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and  
309 ecumenism. *J Hered* 86:248–249. <https://doi.org/10.1093/oxfordjournals.jhered.a111573>
- 310 30. Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and  
311 Linux. *Mol Ecol Resour* 8:103–106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- 312 31. Rice WR (1989) Analyzing Tables of Statistical Tests. *Evolution* 43:223–225.  
313 <https://doi.org/10.1111/j.1558-5646.1989.tb04220.x>
- 314 32. Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying  
315 and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538.  
316 <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- 317 33. Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method  
318 for the analysis of genetically structured populations. *BMC Genet* 11:94. <https://doi.org/10.1186/1471-2156-11-94>
- 320 34. Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*  
321 24:1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- 322 35. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype  
323 data. *Genetics* 155:945–959
- 324 36. Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing  
325 with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801–6.  
326 <https://doi.org/10.1093/bioinformatics/btm233>
- 327 37. Rosenberg NA (2004) distruct: a program for the graphical display of population structure. *Mol Ecol*  
328 *Notes* 4:137–138. <https://doi.org/10.1046/j.1471-8286.2003.00566.x>
- 329 38. Sorenson L, Santini F, Carnevale G, Alfaro ME (2013) A multi-locus timetree of surgeonfishes  
330 (Acanthuridae, Percomorpha), with revised family taxonomy. *Mol Phyl Evol* 68:150–160.  
331 <https://doi.org/10.1016/j.ympev.2013.03.014>

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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<sub>E</sub>*: expected heterozygosity; *H<sub>O</sub>*: observed heterozygosity; *F<sub>IS</sub>*:  
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 <sub>E</sub> /H <sub>O</sub>	F <sub>IS</sub>	Na	H <sub>E</sub> /H <sub>O</sub>	F <sub>IS</sub>	Na	H <sub>E</sub> /H <sub>O</sub>	F <sub>IS</sub>	Na	H <sub>E</sub> /H <sub>O</sub>	F <sub>IS</sub>
<i>Acatri_03083</i>	F: CATTGAGTCACCGCATCCTG R: GCTGAGTTCAGAGCATTGGC	(AC) <sub>13</sub>	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) <sub>14</sub>	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) <sub>14</sub>	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) <sub>18</sub>	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) <sub>18</sub>	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) <sub>15</sub>	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) <sub>15</sub>	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) <sub>13</sub>	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) <sub>14</sub>	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) <sub>16</sub>	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: GGCTAGCTGAGCACATTCAG R: AGCATCGTAGGTATGCGGAG	(GT) <sub>13</sub>	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) <sub>12</sub>	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) <sub>13</sub>	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) <sub>19</sub>	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)
<b>Range</b>	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)
<b>Range</b>	177-187	-	-	90-122	-	-	-	-	90-100	-	-	-	-	104-126
<i>Adus</i> (n=3)	3 (3)	-	4 (2)	2 (3)	-	-	-	-	2 (3)	-	-	-	-	3 (3)
<b>Range</b>	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)
<b>Range</b>	175	-	99	104	-	-	150	-	-	-	85	-	-	110
<i>Anif</i> (n=4)	4 (4)	-	1 (2)	6 (4)	-	-	-	-	-	3 (4)	2 (4)	-	-	6 (4)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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)
<b>Range</b>	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 xanopterus; Cbir : Ctenochaetus birotatus; Ctfla : Ctenochaetus flavicauda; Cstr : Ctenochaetus striatus; Nlit : Naso lituratus.*