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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Abstract

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

Keywords

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

Introduction

Acanthuridae (surgeonfishes, tangs and unicornfishes) are dominant fish taxa in most coral reefs, with an ecological key role in preventing shifts from coral- to algal-dominance following disturbance [1]. Yet, Acanthuridae are under increasing pressure: they are heavily targeted by artisanal fishing (several unicornfish are highly prized in tropical Indo-Pacific fisheries) and/or as ornamental species, being in the top 10 of the most-frequently exported aquarium fish in trade [2–4]. Despite its importance above, and although it is one of the most widespread coral reef fish family in coral reefs, it is one of the least studied in terms of population genetic structure. Only a few studies have investigated phylogeographical patterns and/or population genetic connectivity in these fishes, and for the majority, the genetic variation was inferred using mitochondrial DNA (mtDNA) markers [5–13]. Only a limited number of studies used nuclear microsatellite markers, despite their high resolving power for detecting divergence [14, 15].

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

The convict surgeonfish *A. triostegus* is found throughout the tropical Indo-Pacific, from South African to Baja Californian reefs. The genetic population structure of *A. triostegus* across its entire range, using either allozymes or mtDNA sequences [13, 25] revealed globally congruent results: 1) a marked genetic differentiation of populations from the Hawaiian archipelago, suggesting biogeographic vicariance as an evolutionary process leading to the differentiation of the *A. triostegus* populations in this archipelago; and 2) a significant correlation between genetic differentiation and geographic distance among the remaining populations, indicative of an isolation by distance. Significant genetic differentiation between the Indian and Pacific Ocean populations was found, though only 8.3% of all pairwise comparisons were significant [13]. In addition, no significant differentiation was found across the East Pacific Barrier [6], suggesting a great dispersal potential of this species. Nevertheless, one discrepancy among nuclear and mitochondrial markers remains with the Marquesas population being as much differentiated than the Hawaiian archipelago from the rest of the Pacific populations based on allozymes [25] but not based on mtDNA sequences [6, 12, 13]. This incongruity calls for additional type of markers to be used.

Here we report the development of fourteen microsatellite markers whose power to detect genetic subdivision are tested across four populations sampled across the distribution range of the species. In addition we tested cross-amplification on thirteen congeners to investigate their potential to be used more widely within the Acanthuridae family.

Material and Methods

Microsatellite library development and primer selection

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

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

Polymorphism and cross‐amplification testing

The final set of 14 microsatellite loci selected were further characterized by genotyping individuals sampled in four locations: three islands of the Pacific Ocean–Moorea in French Polynesia, Grande Terre in New Caledonia, and Loloata Island in Papua New Guinea and one island in the Western Indian Ocean, Mayotte (Table 1). Genomic DNA was isolated from fin clips using Gentra Puregene Tissue Kit (Qiagen). PCR reactions were performed using Type-It Microsatellite (Qiagen) in two distinct multiplexes of 5 µl final volume containing 1X Master Mix, 0.5X of Q-solution, 0.1 µM of each primer (fluorescent-labeled forward primer 6-FAM, PET, NED or VIC) and 50 to 150 ng of DNA template (Table 1). All PCRs were conducted in GeneAMP PCR System 9700 (Applied Biosystems) and a unique program was used to amplify the two multiplexes, consisting of 5 min at 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. Fluorescent PCR fragments were visualized on an ABI 3130XL Genetic Analyser (Applied

Biosystems) with GS-500-LIZ (Applied Biosystems). Alleles were sized using GeneMapper® (Applied Biosystems).

Cross-species amplification was tested on 13 species of 3 genus of Acanthuridae sampled in New Caledonia (*A. albipectoralis*, *A. blochii*, *A. dussmieri*, *A. nigricauda*, *A. nigrofuscus*, *A. olivaceus*) and in Moorea (*A. nigricans*, *A. pyroferus*, *A. xanteptorus*, *Ctenochaetus birotatus*, *C. flavicauda*, *C. striatus* and *Naso lituratus*). PCR were conducted in the exact same conditions as described above. We will then only report the number of loci that amplified, the number of alleles observed for each loci and the size range of the alleles.

Data analysis for Acanthurus triostegus

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

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

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

Results and discussion

Characterization of microsatellite loci

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

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

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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Compliance with Ethical Standards:

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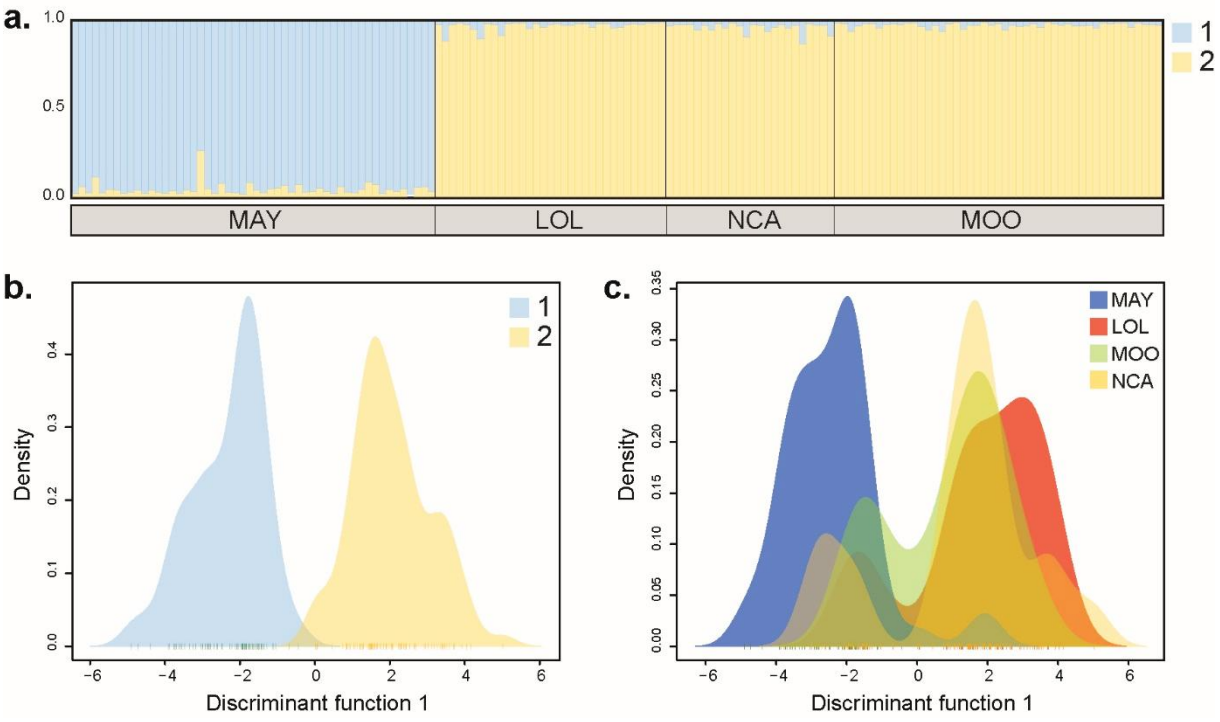

Figure 1: Population differentiation of *A. triostegus* populations based on the analysis of 14 microsatellite loci. STRUCTURE assignment plot showing individual's posterior probabilities of membership to each of the two clusters (**a**). DAPC scatter plots of the first discriminant function of the Principal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

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 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 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}: Weir & Cockerham's (1984) inbreeding coefficient. * = significant after standard Bonferroni correction.

					MOO (*N*=47)			NCA (*N*=24)			LOL (*N*=33)			MAY (*N*=52)		
	Primer sequence 5'-3'	**Repeat array**	***Dye (Mix)***	***Size***	***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}
Acatri_03083	F: CATTGAGTCACCGCATCCTG	$(AC)_{13}$	VIC (2)	161-199	15	0.826	0.032	10	0.827	0.114	8	0.828	0.173	12	0.770	0.011
	R: GCTGAGTTCAGAGCATTGGC					0.809			0.750			0.697			0.769	
Acatri_04614	F: TCAGTGCTGCTGTGAATTGG	$(TG)_{14}$	VIC (1)	134-160	13	0.866	-0.043	12	0.880	-0.015	10	0.846	0.012	12	0.843	-0.058
	R: CTCATGCACAAACACAAGAC					0.913			0.913			0.849			0.900	
Acatri_05455	F: ATACGGACACACAAGTGGGC	$(CA)_{14}$	PET (2)	83-161	22	0.921	0.022	16	0.920	0.062	18	0.905	0.011	14	0.760	-0.025
	R: AGTTTAATTGGTGGCGATGAC					0.911			0.889			0.909			0.787	
Acatri_09735	F : TGTCTATTGTTTTGGACAAGGAGC	$(GT)_{18}$	NED (2)	98-140	21	0.910	0.031	16	0.913	0.077	20	0.912	0.085	24	0.906	0.140
	R: TGGTCCAACCTGAGACAGC					0.891			0.864			0.849			0.789	
Acatri_09917	F: GTGCTCTCAAAGACACAGCC	$(TCTG)_{18}$	NED (2)	190-302	20	0.925	0.137	17	0.927	0.382*	21	0.941	0.210*	20	0.932	0.173*
	R: CATGCCCCATTCGACAAAAC					0.810			0.591			0.758			0.780	
Acatri_10969	F: GGAGCAAATACGAGCGAGTG	$(TG)_{15}$	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
	R: AAGGACGTAGTCAGCACACC					0.894			0.750			0.879			0.846	
Acatri_13144	F: TCTGTTTAAATGCACAAACGC	$(CA)_{15}$	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
	R: GTGTGTCTCCAGATCCAGGC					0.578			0.667			0.758			0.745	
Acatri_13915	F: CAGTCTGCTGAACCTCCTCC	$(AC)_{13}$	PET (1)	90-130	13	0.829	0.523*	10	0.716	0.103	13	0.863	0.045	22	0.919	0.237*
	R: TCGAATCAATCTGTGCGTGC					0.409			0.667			0.839			0.711	
Acatri_14579	F: ACACCAGCACGTCTAGGAAG	$(CA)_{14}$	VIC (1)	86-120	13	0.757	0.092	10	0.804	-0.067	12	0.793	0.060	11	0.468	0.065
	R: ACTGCTGGATAACAGTGTGTG					0.696			0.875			0.758			0.442	
Acatri_15132	F: GAGCTTGACCTACATGTGCC	$(TG)_{16}$	NED (1)	86-124	11	0.791	-0.028	8	0.728	0.276	8	0.752	0.088	11	0.745	0.080
	R: ATCACTTCTCCTGCGTGGAC					0.822			0.542			0.697			0.692	
Acatri_15723	F: GGCTAGCTGAGCACATTCAG	$(GT)_{13}$	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
	R: AGCATCGTAGGTATGCGGAG					0.778			0.792			0.788			0.789	
Acatri_16496	F: ATCCTCTGACAATAGGCCCG	$(GT)_{12}$	PET (1)	146-170	10	0.817	0.066	8	0.817	-0.089	11	0.820	-0.020	14	0.867	0.012
	R: TGCAGACACTATGTAGTCCACC					0.773			0.909			0.849			0.865	
Acatri_17233	F: GGGCTCGTTTATCTGCAAGG	$(GT)_{13}$	NED (1)	126-166	17	0.912	-0.006	11	0.891	0.183	16	0.888	0.025	17	0.908	-0.068
	R: GTAAGTGATCTCGGTTAGATGC					0.929			0.750			0.879			0.979	
Acatri_18344	F: TCAGCCAGCCGAATCTGAAC	$(TG)_{19}$	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
	R: CTCACCAAGCCATGTTAGCC					0.809			0.750			0.758			0.923	
over all loci					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.

	Acatri_ 03083	***Acatri_ 04614***	***Acatri_ 05455***	***Acatri_ 09735***	***Acatri_ 09917***	***Acatri_ 10969***	***Acatri_ 13144***	***Acatri_ 13915***	***Acatri_ 14579***	***Acatri_ 15132***	***Acatri_ 15723***	***Acatri_ 16496***	***Acatri_ 17233***	***Acatri_ 18344***
***Aalb* (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
***Ablo* (n=5)**	5 (5)	-	-	5 (4)	-	-	-	-	4 (3)	-	-	-	-	3 (5)
Range	177-187	-	-	90-122	-	-	-	-	90-100	-	-	-	-	104-126
***Adus* (n=3)**	3 (3)	-	4 (2)	2 (3)	-	-	-	-	2 (3)	-	-	-	-	3 (3)
Range	177-189	-	93-111	104-106	-	-	-	-	80-82	-	-	-	-	116-120
***Anic* (n=1)**	1 (1)	-	1 (1)	1 (1)	-	-	1 (1)	-	-	-	1 (1)	-	-	1 (1)
Range	175	-	99	104	-	-	150	-	-	-	85	-	-	110
***Anif* (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
***Anig* (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
***Aoli* (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
***Apyr* (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
***Axan* (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
***Cbir* (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
***Ctfla* (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
***Cstr* (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
***Nlit* (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 dussmieri; Anic: Acanthurus nigricauda; Anif: Acanthurus nigrofuscus; Anig : Acanthurus nigricans; Aoli: Acanthurus olivaceus; Apyr : Acanthurus pyroferus; Axan : Acanthurus xanteptorus; Cbir : Ctenochaetus birotatus; Ctfla : Ctenochaetus flavicauda; Cstr : Ctenochaetus striatus; Nlit : Naso lituratus.