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Marine and Freshwater Research

Supplementary Material

Molecular ecology of the Javanese ricefish, *Oryzias javanicus* (Bleeker): genetic divergence along the Indonesian Archipelago

Eko Hardianto^{A,}, Mio Hirayama^B, Diah Permata Wijayanti^C, and Hideyuki Imai^B*

^ADepartment of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Sleman, Yogyakarta 55223, Indonesia.

^BLaboratory of Marine Biology and Coral Reef Studies, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

^CDepartment of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, Central of Java 50275, Indonesia.

*Correspondence to: Eko Hardianto Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Sleman, Yogyakarta 55223, Indonesia Email: ekohardianto@ugm.ac.id

Table S1 Detail of one mtDNA CR primer and four microsatellite loci used in this study.

Primer Name	Primer sequence (5'-3')	Repeat	Allele size range (bp)	Reference
12sarH	ATAGTGGGGTATCTAATCCCAGTT		692	Palumbi <i>et al.</i> 1991
Pro-goby	CCACCBCTRRCTCCCAAAGC			Tomita <i>et al.</i> 2016
Orla 2-91	VIC- TGGATCAGYATTAGGCTCATCGGTTG GAGGTTTGACATTCC	(GT) ₇	59	Gotoh <i>et al.</i> 2013
Orla 13-81	6-FAM- CAGCAGGGCTTTAATTATGAGGTAGG AGTTGCATAACWAGAC	(CA) ₈	59	
Orla 16-32	VIC- TAATTGAATGGGCATGTGCCTAGCAA GGAGAGCCTGATGC	(GT) ₅	59	
Orla 9-204	VIC- TATGCTGACCTGCTGTGACCCGCCAG ATGTCGCAAATAC	(CA) ₅ , (CA) ₁₀	59	

Table S2 Spatial distribution of mtDNA CR haplotypes among *Oryzias javanicus* from seven sampling sites.

Haplotype number	Location						
	PAN	BAN	TAN	BAL	LOM	BIM	KON
1	45						
2	1						
3	1						
4	1						
5	2						
6		34		31			
7		6		1			
8		1					
9		1					
10		1		2			
11		1					
12		1					
13		1		1			
14		1		1	3		19
15			30				
16			8				
17			1				
18			4				
19			5				
20			1				
21			1				
22			1				
23			1		1		
24				1			
25				1			
26				1			
27				1			
28				4	16		
29				1			
30				1	2		
31				1	4		
32				1			
33					5		
34					1		
35					1		
36					1		
37					1		
38					1		
39					1		
40					1		
41					1		
42					1		
43					1		

Haplotype number	Location						
	PAN	BAN	TAN	BAL	LOM	BIM	KON
44					1		
45					1		
46						28	
47						12	
48						5	
49						1	
50						1	
51							1
52							14
53							12
54							1
55							1

Location abbreviations are shown in Table 1.

Table S3 Nei's estimation of heterozygosity in *Oryzias javanicus*.

Locus Name	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'	Gis
Locus 2-91	0.173	0.217	0.407	0.190	0.222	0.439	0.468	0.506	0.200
Locus 13-81	0.112	0.374	0.600	0.226	0.264	0.638	0.377	0.413	0.702
Locus 16-32	0.147	0.181	0.324	0.143	0.166	0.348	0.440	0.479	0.188
Locus 9-204	0.416	0.453	0.659	0.205	0.240	0.693	0.312	0.346	0.082
Overall	0.212	0.306	0.498	0.191	0.223	0.529	0.384	0.421	0.308

Table S4 Locus-by-locus summary statistics for each sampled *Oryzias javanicus* population.

Location (<i>n</i>)	Locus				Mean
	Orla 2-91	Orla 13-81	Orla 16-32	Orla 9-204	
Pandeglang (40)					
N_a	2	2	2	4	2.5
AR	2	2	2	4	2.5
H_o	0.438	0.031	0.031	0.438	0.234
H_e	0.508	0.032	0.032	0.439	0.252
Fis	0.141	<0.0001	<0.0001	0.005	
P_{HW}	0.492	1	1	0.647	
Null alleles	No	No	No	No	
Banyuwangi (40)					
N_a	3	3	3	5	3.5
AR	2.762	2.762	2.762	4.592	3.211
H_o	0.025	0.025	0.025	0.225	0.075
H_e	0.073	0.073	0.073	0.211	0.108
Fis	0.664	0.664	0.664	-0.068	
P_{HW}	0.012	0.013	0.013	1	
Null alleles	No	No	No	No	
Tanah Bumbu (40)					
N_a	3	2	3	5	3.25
AR	2.962	2	3	4.986	3.237
H_o	0.125	<0.0001	0.625	0.5	0.313
H_e	0.164	0.425	0.562	0.576	0.432
Fis	0.244	1	-0.113	0.134	
P_{HW}	0.027	<0.0001	0.051	0.234	
Null alleles	No	No	No	No	
Bali Island (40)					
N_a	3	2	5	3	3.25
AR	2.961	2	4.761	3	3.181
H_o	0.5	<0.0001	0.175	0.35	0.144
H_e	0.143	0.353	0.293	0.43	0.305
Fis	0.653	1	0.407	0.189	
P_{HW}	0.0005	<0.0001	0.00008	0.138	
Null alleles	No	No	No	No	
Lombok Island (40)					
N_a	4	3	5	7	4.75
AR	3.762	2.8	4.4	3	3.491
H_o	0.275	0.025	0.075	0.425	0.2
H_e	0.321	0.386	0.209	0.507	0.355
Fis	0.144	0.936	0.644	0.164	
P_{HW}	0.037	<0.0001	<0.0001	0.0216	
Null alleles	No	No	No	No	

Location (<i>n</i>)	Locus				Mean
	Orla 2-91	Orla 13-81	Orla 16-32	Orla 9-204	
Bima (40)					
<i>N_a</i>	2	7	4	4	4.25
<i>AR</i>	2	6.955	3.992	3.992	4.235
<i>H_o</i>	0.125	0.6	0.45	0.45	0.392
<i>H_e</i>	0.119	0.772	0.398	0.398	0.429
Fis	-0.054	0.225	-0.135	-0.135	
<i>P</i> HW	1	<0.0001	0.834	0.834	
Null alleles	No	No	No	No	
South Konawe (40)					
<i>N_a</i>	3	3	2	3	2.75
<i>AR</i>	2.8	2.999	1.999	3	
<i>H_o</i>	0.175	0.1	0.1	0.525	0.225
<i>H_e</i>	0.184	0.553	0.096	0.609	0.361
Fis	0.052	0.821	0.04	0.14	
<i>P</i> HW	0.104	<0.0001	1	0.564	
Null alleles	No	No	No	No	

P-values were set to test for departures from Hardy–Weinberg equilibrium and significant values are highlighted in bold.

The potential presence of null alleles was tested using Micro-Checker (ver. 2.3.3, see

<https://micro-checker.software.informer.com/2.2/>). Sample size, number of individuals; *N_a*, number of alleles; *H_o*, observed heterozygosity and potential for null alleles; *H_e*, expected heterozygosity and potential for null alleles.

Table S5 Allele frequencies (%) of four microsatellite loci in *Oryzias javanicus*.

Locus	Location						
	PAN	BAN	TAN	BAL	LOM	BIM	KON
Orla 2-91							
133	50	96.3	6.3	92.5	81.3	93.8	90
134	-	2.5	2.5	5	-	-	-
135	50	1.3	91.3	2.5	15	6.3	8.8
137	-	-	-	-	-	-	1.3
138	-	-	-	-	2.5	-	-
142	-	-	-	-	1.3	-	-
Orla 13-81							
175	-	-	-	-	-	-	5
177	-	-	-	-	-	35	-
178	-	-	-	-	-	27.5	-
181	-	1.3	-	-	-	-	-
182	-	2.5	70	22.5	75	3.8	47.5
183	98.4	96.3	30	77.5	23.8	7.5	47.4
184	-	-	-	-	-	16.3	-
185	1.6	-	-	-	-	7.5	-
186	-	-	-	-	1.3	2.5	-
Orla 16-32							
238	-	-	-	-	1.2	-	-
240	-	-	38.8	-	-	-	-
242	98.4	96.3	7.5	85	90	95	95
243	-	2.5	-	5	7.5	-	-
244	1.6	1.3	53.7	7.5	1.3	5	5
246	-	-	-	2.5	-	-	-
Orla -2-4							
268	-	-	-	-	-	15	-
272	-	-	3.8	-	2.5	-	-
274	4.7	3.8	7.5	21.3	20	5	12.5
276	14.1	1.3	60	-	3.8	-	43.8
278	73.4	88.8	25	72.5	67.5	3.8	43.8
279	-	-	-	-	2.5	-	-
280	7.8	5	-	6.3	1.3	-	-
282	-	-	3.8	-	2.5	-	-
284	-	1.3	-	-	-	-	-
286	-	-	-	-	-	76.3	-

Table S6 Statistic analysis of neutrality tests and mismatch distribution of seven localities of *Oryzias javanicus*.

Locations	Neutrality tests				Demographic parameters			Test of goodness of fit	
	D	$P(D)$	F_s	$P(F_s)$	τ	θ_0	θ_1	Hri	$P(\text{Hri})$
PAN	-2.1251	0.002	-2.6754	0.020	1.730	2.102	31.875	0.014	0.700
BAN	-1.5307	0.040	-5.9639	0.001	0.138	0.982	10.755	0.061	0.640
TAN	-0.9602	0.183	-2.3336	0.117	2.125	<0.001	2989.98	0.042	0.010
BAL	-0.6880	0.286	-2.1729	0.198	2.125	0.450	3469.98	0.032	0.049
LOM	-0.7269	0.253	-6.9726	0.006	8.488	0.003	10.348	0.009	1.000
BIM	0.8364	0.810	5.1443	0.957	4.429	0.024	4.791	0.064	0.280
KON	0.0409	0.519	1.5355	0.769	5.449	0.017	55.056	0.012	0.440

D , Tajima's D ; $P(D)$, P -value of Tajima's D ; F_s , Fu's F_s ; $P(F_s)$, P -value of Fu's F_s ; τ , τ value of mismatch distribution; θ_0 , population size before expansion; θ_1 , population size after expansion; Hri, Harpending's raggedness index; $P(\text{Hri})$, P -value of raggedness index. Location abbreviations are shown in Table 1.

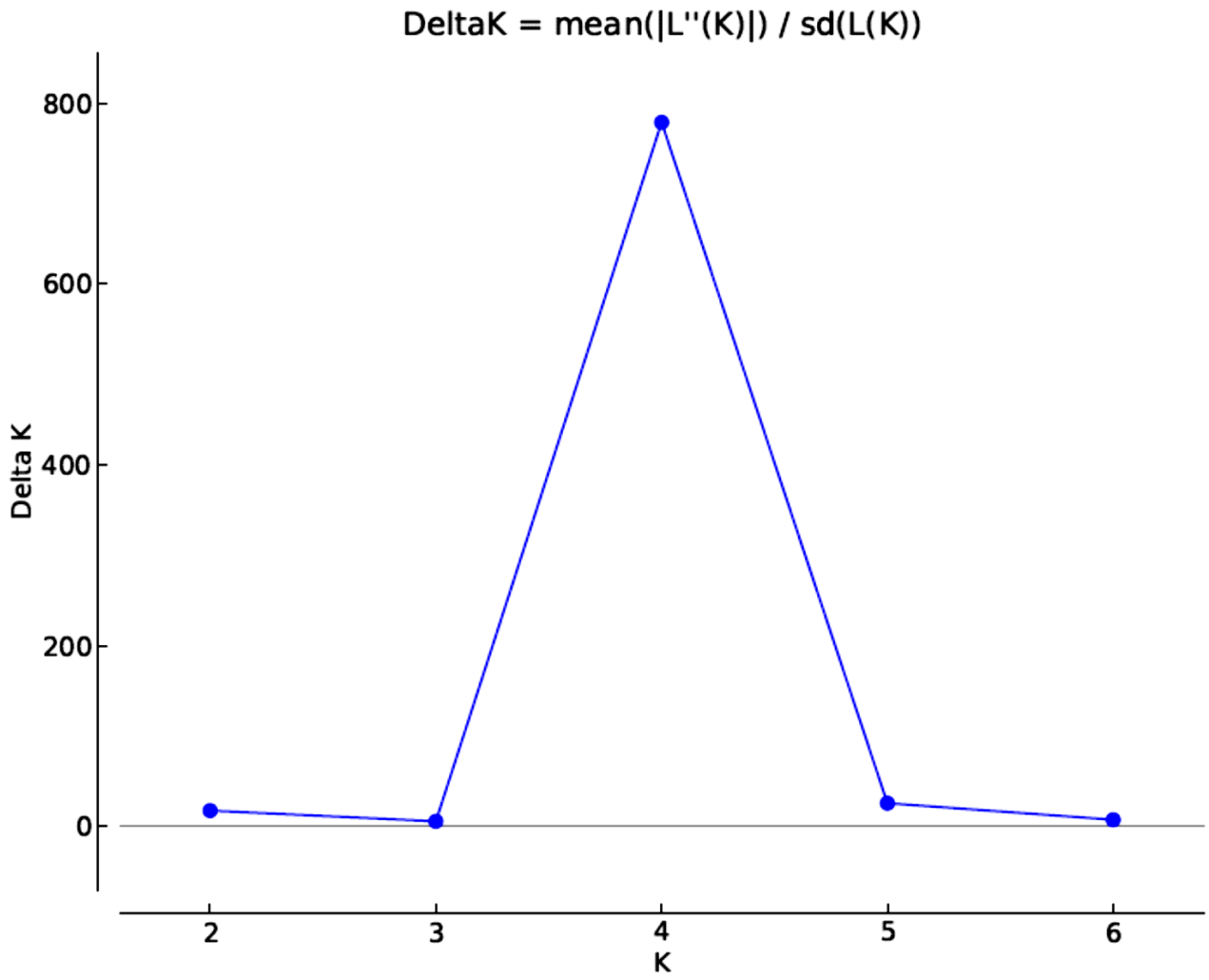


Fig. S1 STRUCTURE HARVESTER output for *Oryzias javanicus* showing results of Evanno's ΔK method of establishing the most supported value of K (see Evanno *et al.* 2005 for details). All loci included.

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