APPENDIX A

Table 9 Abbreviation of measurable characters in this study

Type of measurement	Morphometric characteristics	Abbreviation
General characters measurement	Total length	TL
	Standard length	SL
	Fork length	FL
	Pre-anal length	PAL
	Pre-dorsal length	PDL
	Pre-pelvic length	PPVL
	Pre-pectoral length	PPTL
	Body depth	BD
	Head length	HL
	Eye diameter	ED
	Pre-orbital length	POL
	Pecteral fins length	PTFL
	Pevic fins length	PVFL
New characters measurement	Anterior tip of the snout on the upper jaw to posterior nostril	1-2
	Anterior tip of the snout on the upper jaw to origin of spinous dorsal fin	1-4
	Anterior tip of the snout on the upper jaw to origin of pelvic fin	1-3
	Posterior nostril to origin of spinous dorsal fin to origin of spinous dorsal fin	2-4
	Posterior nostril to origin of pelvic fin to origin of pelvic fin	2-3

Table 9 Abbreviation of measurable characters in this study (continued)

Type of measurement	Morphometric characteristics	Abbreviation
New characters measurement (continued)	Origin of spinous dorsal fin to original of soft dorsal fin	4-5
	Origin of spinous dorsal fin to origin of anal fin	4-10
	Origin of pelvic fin to original of soft dorsal fin	3-5
	Origin of pelvic fin to origin of anal fin	3-10
	Original of soft dorsal fin to origin of anal fin	5-10
	Original of soft dorsal fin to end of soft dorsal fin	5-6
	Original of soft dorsal fin to insertion of anal fin	5-9
	End of soft dorsal fin to origin of anal fin	6-10
	Origin of anal fin to insertion of anal fin	10-9
	End of soft dorsal fin to insertion of anal fin	6-9
	End of soft dorsal fin to insertion of 1st dorsal caudal fin ray	6-7
	End of soft dorsal fin to insertion of 1st ventral caudal fin ray	6-8
	Insertion of 1st dorsal caudal fin ray to insertion of anal fin	7-9
	Insertion of 1st dorsal caudal fin ray to insertion of 1st ventral caudal fin ray	7-8
	Insertion of anal fin to insertion of 1st ventral caudal fin ray	9-8

Note: 10 landmarks of new characters measurement refer to: (1) anterior tip of the snout on the upper jaw; (2) Posterior nostril which is far from upper jaw than anterior nostril; (3) origin of pelvic fin; (4) origin of spinous dorsal fin; (5) original of soft dorsal fin; (6) end of soft dorsal fin (7) insertion of 1st dorsal caudal fin ray. (8) insertion of 1st ventral caudal fin ray; (9) insertion of anal fin (10) origin of anal fin.

APPENDIX B

Table 10 Pair-wise Correlation between adult's characters

		POL	PVFL	1-2	1-4
POL	corelation coefficient	1.000			
	Sig. (2-tailed)	-			
	N	33			
PVFL	corelation coefficient	-0.131	1.000		
	Sig. (2-tailed)	0.469	-		
	N	33	33		
1-2	corelation coefficient	0.381	0.100	1.000	
	Sig. (2-tailed)	0.029*	0.581	-	
	N	33	33	33	
1-4	corelation coefficient	-0.335	0.323	-0.044	1.000
	Sig. (2-tailed)	0.057	0.066	0.806	-
	N	33	33	33	33

Only the distance from anterior tip of the snout on the upper jaw to posterior nostril (1-2) of adult sample was correlated with pre-orbital length (POL) and this two characters were not related to another (Table 10).

In the juvenile samples, many characters related with each other, that were distance between Insertion of anal fin to insertion of 1st ventral caudal fin ray (9-8), Original of soft dorsal fin to origin of anal fin (5-10), posterior nostril to origin of pelvic fin to origin of pelvic fin (2-3), end of soft dorsal fin to origin of anal fin (6-10), insertion of 1st dorsal caudal fin ray to insertion of 1st ventral caudal fin ray (7-8), origin of spinous dorsal fin to origin of anal fin (4-10), original of soft dorsal fin to insertion of anal fin (5-9), end of soft dorsal fin to insertion of anal fin (6-9), origin of pelvic fin to original of soft dorsal fin (3-5), origin of spinous dorsal fin to original of soft dorsal fin (4-5), pre-pelvic length (PPVL) and pre-pectoral length (PPTL) (Table 11). However, these characters were significant difference by mean and apply to principle component analysis (PCA)

Table 11 Pair-wise Correlation between juvenile's characters

		TL	PPVL	PPTL	ED	4-5	4-10	3-5	5-10	5-9	6-9	7-8	9-8	2-3	6-10
TL	CC	1.000													
	Sig.	-													
	N	31													
PPVL	CC	0.213	1.000												
	Sig.	0.251	-												
	N	31	31												
PPTL	CC	0.334	0.499**	1.000											
	Sig.	0.066	0.004	-											
	N	31	31	31											
ED	CC	0.159	0.186	-0.042	1.000										
	Sig.	0.394	0.316	0.823	-										
	N	31	31	31	31										
4-5	CC	-0.118	-0.223	-0.216	0.019	1.000									
	Sig.	0.528	0.229	0.242	0.917	-									
	N	31	31	31	31	31									
4-10	CC	-0.065	-0.144	-0.170	0.207	0.354	1.000								
	Sig.	0.726	0.440	0.361	0.264	0.051	-								
	N	31	31	31	31	31	31								
3-5	CC	-0.174	-0.365*	-0.466**	0.091	0.373*	0.351	1.000							
	Sig.	0.351	0.044	0.008	0.627	0.039	0.053	-							
	N	31	31	31	31	31	31	31							
5-10	CC	-0.180	0.011	-0.195	0.289	0.269	0.690**	0.294	1.000						
	Sig.	0.333	0.954	0.294	0.115	0.143	0.000	0.109	-						
	N	31	31	31	31	31	31	31	31						

Table 11 Pair-wise Correlation between juvenile's characters (continued)

		TL	PPVL	PPTL	ED	4-5	4-10	3-5	5-10	5-9	6-9	7-8	9-8	2-3	6-10
5-9	CC	-0.106	-0.181	-0.238	0.298	-0.002	0.570*	0.050	0.734**	1.000					
	Sig.	0.570	0.329	0.197	0.103	0.992	0.001	0.788	0.000	-					
	N	31	31	31	31	31	31	31	31	31					
6-9	CC	-0.233	-0.163	-0.204	0.015	0.306	0.513**	0.286	0.491**	0.353	1.000				
	Sig.	0.208	0.380	0.271	0.936	0.094	0.003	0.119	0.005	0.051	-				
	N	31	31	31	31	31	31	31	31	31	31				
7-8	CC	-0.067	0.141	-0.169	0.292	0.255	0.470*	0.478**	0.357*	0.235	0.451*	1.000			
	Sig.	0.721	0.451	0.364	0.111	0.166	0.008	0.007	0.049	0.203	0.011	-			
	N	31	31	31	31	31	31	31	31	31	31	31			
9-8	CC	-0.045	-0.039	-0.320	0.182	0.324	0.594**	0.411*	0.645**	0.496**	0.546**	0.505**	1.000		
	Sig.	0.809	0.837	0.079	0.3226	0.075	0.000	0.022	0.000	0.005	0.001	0.004	-		
	N	31	31	31	31	31	31	31	31	31	31	31	31		
2-3	CC	0.102	0.612**	0.415*	0.187	-0.376*	-0.022	-0.294	0.081	-0.038	-0.084	0.267	-0.079	1.000	
	Sig.	0.585	0.000	0.020	0.313	0.037	0.907	0.108	0.666	0.838	0.653	0.146	0.671	-	
	N	31	31	31	31	31	31	31	31	31	31	31	31	31	
6-10	CC	-0.218	-0.032	0.026	0.226	0.378*	0.461**	0.146	0.574**	0.349	0.497**	0.310	0.303	-0.049	1.000
	Sig.	0.239	0.865	0.888	0.222	0.036	0.009	0.432	0.001	0.054	0.004	0.089	0.098	0.792	-
	N	31	31	31	31	31	31	31	31	31	31	31	31	31	31

Note: all abbreviations was detail in (Appendix A)

CC is corelation coefficient

Sig. is significant value (2-tailed)

* indicated statistical significance when p-value < 0.05

** indicated statistical significance when p-value < 0.01

APPENDIX C

Table 12 Characters differentiation of Adult fish between Gulf of Thailand and Andaman Sea

Measurable characters		AN		GT		F value	df	Mann-Whitney U value	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
General characters	TL	26 ±2.4	1.2±0.0	29.9±4.0	1.2±0.0	0.363	32	-	0.551
	FL	27.7±2.3	1.1±0.0	28.7±3.8	1.1±0.0	1.519	32	-	0.227
	PAL	15.7±2.4	0.6±0.1	16.8±2.1	0.7±0.0	-	-	0.6864	0.407
	PDL	9.1±1.0	0.4±0.0	9.4±1.4	0.4±0.0	0.594	32	-	0.447
	PPVL	9.3±1.7	0.4±0.1	9.0±1.3	0.4±0.0	-	-	0.825	0.177
	PPTL	8.1±0.6	0.3±0.0	8.4±1.2	0.3±0.0	0.044	32	-	0.836
	BD	8.1±0.8	0.3±0.0	8.9±1.7	0.4±0.0	0.045	32	-	0.833
	HL	8.7±0.8	0.4±0.0	9.2±1.2	0.4±0.0	2.253	32	-	0.143
	ED	2.1±0.2	0.1±0.0	1.9±0.2	0.1±0.1	2.387	32	-	0.132
	POL	2.6±0.3	0.1±0.0	3.1±0.4	0.1±0.0	14.243	32	-	0.001**
	PVFL	4.8±0.4	0.2±0.0	5.2±0.9	0.2±0.0	13.776	32	-	0.001**
New characters	1-2	1.8±0.2	0.1±0.0	2.1±0.4	0.1±0.0	5.801	32	-	0.022*
	1-4	10.3±0.9	0.4±0.0	10.3±1.3	0.4±0.0	-	-	5.398	0.020*
	2-4	8.6±0.9	0.4±0.0	8.7±1.2	0.3±0.0	3.036	32	-	0.091
	2-3	8.4±0.7	0.3±0.0	8.8±1.2	0.3±0.0	0.71	32	-	0.406
	4-5	7.3±0.6	0.3±0.0	7.6±1.0	0.3±0.0	-	-	0.016	0.898
	4-10	11.2±1.1	0.5±0.0	11.7±1.5	0.5±0.0	0.16	32	-	0.692
	3-5	10.4±1.1	0.4±0.0	11.0±1.4	0.4±0.0	0.581	32	-	0.452
	3-10	7.4±0.9	0.3±0.0	7.8±1.0	0.3±0.0	0.549	32	-	0.464
	5-10	7.6±0.7	0.3±0.0	8.0±1.0	0.3±0.0	0.918	32	-	0.345
	5-6	4.4±0.5	0.2±0.0	4.8±0.8	0.2±0.0	3.358	32	-	0.077
	5-9	7.0±0.7	0.3±0.0	7.4±1.0	0.3±0.0	1.815	32	-	0.188
	6-10	6.5±0.5	0.3±0.0	6.7±0.7	0.3±0.0	0.231	32	-	0.634
	10-9	3.3±0.2	0.1±0.0	3.4±0.4	0.1±0.0	0.022	32	-	0.882

Table 12 Characters differentiation of Adult fish between Gulf of Thailand and Andaman Sea (Continued)

Measurable characters		AN		GT		F value	df	Mann-Whitney U value	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
New characters	6-9	4.0±0.4	0.2±0.0	4.3±0.6	0.2±0.0	-	-	0.203	0.652
	6-7	3.7±0.5	0.2±0.0	4.1±1.3	0.2±0.1	-	-	0.06	0.806
	6-8	5.1±0.4	0.2±0.0	5.1±0.7	0.2±0.0	2.804	32		0.104
	7-9	5.6±1.2	0.2±0.0	5.9±0.9	0.2±0.0	-	-	0.657	0.418
	7-8	3.7±0.4	0.2±0.0	3.9±1.2	0.2±0.0	0.039	32	-	0.845
	9-8	4.3±0.5	0.2±0.0	4.2±0.8	0.2±0.0	3.331	32	-	0.078

Note: * indicated statistical significance when p-value < 0.05

** indicated statistical significance when p-value < 0.01

Table 13 Characters differentiation of juvenile fish between Gulf of Thailand and Andaman Sea

Measurable characters		AN		GT		F value	df	Mann-Whitney U value	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
General characters	TL	15.8±1.4	1.2±0.0	15.1±1.6	1.2±0.0	4.598	30		0.041*
	FL	15.1±1.3	1.1±0.0	14.4±1.5	1.1±0.0			3.616	0.057
	PAL	8.8±0.9	0.7±0.0	8.3±1.0	0.7±0.0	0.148	30		0.703
	PDL	5.0±0.4	0.4±0.0	4.8±0.5	0.4±0.0	1.911	30		0.177
	PPVL	5.0±0.7	0.4±0.0	5.0±0.6	0.4±0.0	6.804	30		0.014*
	PPTL	4.6±0.5	0.3±0.0	4.6±0.4	0.4±0.0	11.84	30		0.002**
	BD	4.5±0.4	0.3±0.0	4.2±0.5	0.3±0.0			0.35	0.554
	HL	4.9±0.5	0.4±0.0	4.6±0.5	0.4±0.0			2.379	0.123
	ED	1.2±0.1	0.1±0.0	1.1±0.1	0.1±0.0	4.622	30		0.400
	POL	1.5±0.2	0.1±0.0	1.5±0.3	0.1±0.0	1.893	30		0.179
	PVFL	2.8±0.2	0.2±0.0	2.8±0.3	0.2±0.0	1.679	30		0.205

Table 13 Characters differentiation of juvenile fish between Gulf of Thailand and Andaman Sea (Continued)

Measurable characters		AN		GT		F value	df	Mann-Whitney U value	P-value
		Average raw data ±SD	Ratio by SL±SD	Average raw data ±SD	Ratio by SL±SD				
New characters	1-2	1.0±0.1	0.1±0.0	0.9±0.2	0.1±0.0			0.161	0.688
	1-4	5.4±0.5	0.4±0.0	5.0±0.6	0.4±0.0	2.675	30		0.113
	2-4	4.5±0.4	0.3±0.0	4.2±0.4	0.3±0.0	3.085	30		0.090
	2-3	4.6±0.6	0.3±0.0	4.6±0.6	0.4±0.0	8.035	30		0.008**
	4-5	4.1±0.5	0.3±0.0	3.7±0.4	0.3±0.0	5.732	30		0.023*
	4-10	6.1±0.6	0.5±0.0	5.6±0.7	0.4±0.0	6.162	30		0.019*
	3-5	0.8±0.6	0.4±0.0	5.2±0.7	0.4±0.0			9.129	0.002**
	3-10	3.8±0.5	0.3±0.0	3.4±0.5	0.3±0.0	2.599	30		0.118
	5-10	4.4±0.4	0.3±0.0	4.0±0.5	0.3±0.0	10.758	30		0.003**
	5-6	2.5±0.3	0.2±0.0	2.2±0.3	0.2±0.0	2.003	30		0.168
	5-9	3.9±0.3	0.3±0.0	3.5±0.6	0.3±0.0			19.69	0.000**
	6-10	3.8±0.4	0.3±0.0	3.4±0.4	0.3±0.0	5.029	30		0.033*
	10-9	2.0±0.2	0.2±0.0	1.8±0.3	0.1±0.0	1.068	30		0.310
	6-9	2.3±0.3	0.2±0.0	2.0±0.2	0.2±0.0			9.002	0.003**
	6-7	2.1±0.2	0.2±0.0	1.9±0.4	0.1±0.0	1.753	30		0.196
	6-8	2.9±0.4	0.2±0.0	2.6±0.4	0.2±0.0	2.113	30		0.157
	7-9	3.3±0.4	0.2±0.0	3.0±0.5	0.2±0.0	3.614	30		0.067
	7-8	2.0±0.2	0.2±0.0	1.8±0.3	0.1±0.0	4.719	30		0.038*
	9-8	2.5±0.3	0.2±0.0	2.1±0.4	0.2±0.0			8.016	0.005**

Note: * indicated statistical significance when p-value < 0.05

** indicated statistical significance when p-value < 0.01

APPENDIX D

SCREE PLOT OF JUVENILE AND ADULT

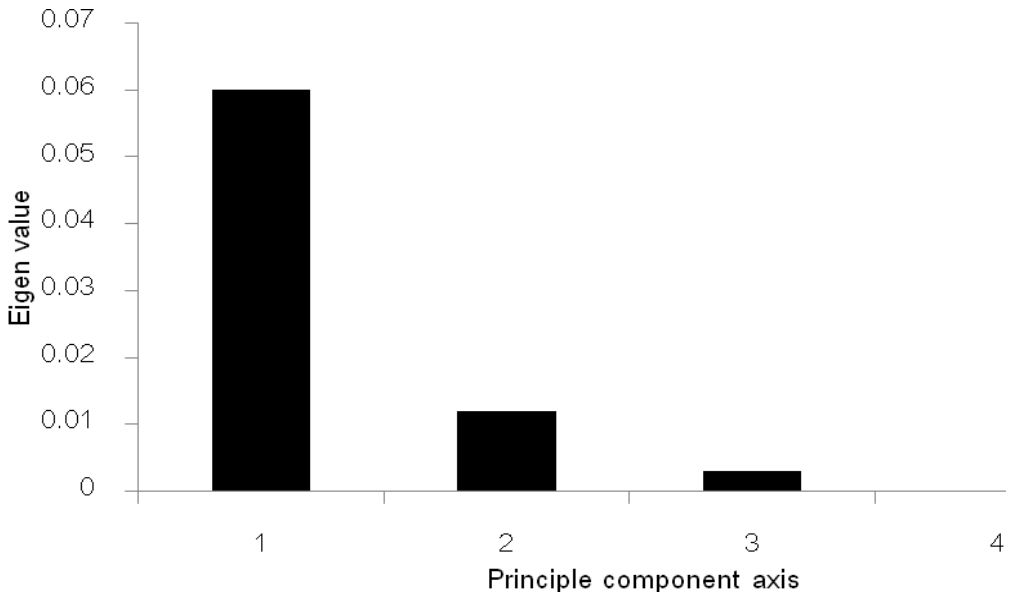

Figure 21 Scree plot of adult samples

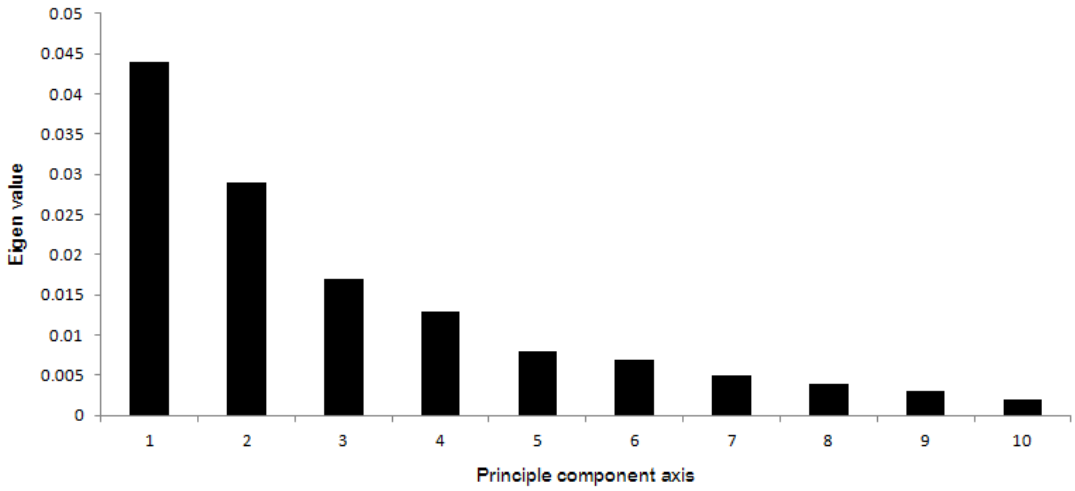

Figure 22 Scree plot of juvenile

APPENDIX E

EXTRACTION OF DNA FROM FIN TISSURE

1. Place tissue that was preserved in acetone on some tissue paper to drain off any excess acetone.

2. Take a small amount of tissue (~ 0.5 cm^2) and cut it as fine as possible with sterile scissors. Transfer the sample into a 1.5 ml microcentrifuge tube.

3. Add 500 µL of TNES buffer and homogenize with a homogenizer

4. Add 17.5 µL of Proteinase-K (20mg/ml). Mix the sample by inverting the tube several times.

5. Incubate the sample at least 2 hr. at 55 °C in water bath, occasionally mix the sample by inverting the tube – be careful while warming the lids can be loose.

6. Add 200 µL of Phenol and 200 µL of CIA (Chloroform –Isoamyl alcohol 24:1), then slowly invert tube to mix the solution, vibrate too roughly may damage DNA.

7. Centrifuge the samples at 12000 rpm for 10 minutes at room temperature.

8. Transfer the upper layer to a new label tube and add 400 µL of CIA. Centrifuge again at 12000 rpm for 10 minutes

9. Transfer the upper layer to a new tube, make sure that don't transfer any Phenol or CIA solution to the new tube.

10. Add cold 100% ethanol into the tube until the volume is up to1500 µL and gently mix by inverting the tube a couple of time – the write pellet DNA should be precipitated out of the solution.

11. Centrifuge the samples at 12000 rpm for 10 minutes at room temperature. Remove ethanol by pouring or pipetting.

12. Washthe DNA pellet again by adding 500 µL of 70% ethanol into tube, invert the tube a couple of time, and then centrifuge the samples at 12000 rpm for 5 minutes. Remove ethanol by pouring or pipetting.

13. Leave the sample to air dry – usually 10-30 min depending upon the temperature and thickness of DNA pellet.

14. Add 100 µL of sterile distilled water (DI) into dried DNA pellet and vibrate until DNA pellet dissolve.

15. Store DNA at -30 °C in a freezer.

APPENDIX F

Table 14 Microsatellite markers of *L. russelli* developed by Guo *et al*. (2007)

Locus	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	Repeat motif	N_a	HWE *P*	H_E	H_O
Lru001 Forward	TCCCTCTGTTGTTGAAAG	149	58	$(CA)_{30}$	14	0.083	0.95	0.93
Lru001 Reverse	CCTGATCTCGATAGTGCC							
Lru002 Forward	AGGTCTCCCCTGCAACAG	196	58	$(AC)_{22}$	10	0.509	0.90	0.89
Lru002 Reverse	CACAACCCCACTTCAAAA							
Lru003 Forward	GCATCTGCCTGGGAACTT	158	60	$(AC)_{43}$	13	0.196	0.95	0.93
Lru003 Reverse	GCAAGAGGCTGTCGGTGT							
Lru010 Forward	GCAAACGGAGGAAACAAA	153	60	$(CA)_{28}$	14	0.075	1.00	0.94
Lru010 Reverse	CTGAAGCTCGGATGAGGA							
Lru011 Forward	TGTGCTGCTGAGGACTGA	165	60	$(AC)_{18}$	14	0.992	1.00	0.93
Lru011 Reverse	CACCCTGCGTGCGTAAGT							
Lru012 Forward	ATGTTGGCTGAATCGTAG	250	52	$(AC)_{32}$	12	0.059	0.90	0.92
Lru012 Reverse	GACCAGGTCTCCTTGAGGTT							
Lru014 Forward	TGGAGGAAAATCTGTCTA	216	56	$(AC)_{19}$	16	0.498	1.00	0.95
Lru014 Reverse	AGAGTAGCAGGTTTGATG							
Lru025 Forward	ACCACGCTGCACGAGATT	137	66	$(AC)_5(AC)_2$ $(AC)_{10}(AC)_7$	10	0.111	0.79	0.84
Lru025 Reverse	GGCTTATACCGACCCACC							

Table 14 Microsatellite markers of *L. russelli* developed by Guo *et al*. (2007) (continued)

Locus	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	Repeat motif	N_a	HWE *P*	H_E	H_O
Lru030 Forward	TGCCATTCAGTCCCATTA	239	62	$(CT)_{12}(CA)_7(CA)_{27}(AATA)_7$	13	0.239	1.00	0.91
Lru030 Reverse	CCAGTCCACAGTTCACCC							
Lru043 Forward	CACAAATGGGCACAATAA	258	56	$(CACT)_{13}$	10	0.273	0.85	0.88
Lru043 Reverse	GGCAACATGGACGTGTAA							

APPENDIX G

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) PROCESS

The microsatellite markers were amplified, and then polyacrylamide gel electrophoresis (PAGE) was applied to detect the microsatellite alleles in a denaturing polyacrylamind gel . The DNA bands were visualized by silver staining (Caetano-Anollés and Gresshoff, 1994). This staining technique is cheaper, lower time consuming, no need for specific facilities, and safer for health than other stain techniques such as radioactive or fluorescent labeling of nucleotides (Creste *et al*., 2001). This study employed a new polyacrylamide gel electrophoresis (PAGE) process and silver staining techniques adapted from Creste *et al*. (2001).

Gel electrophoresis

- Treat the glass (38.2 x 32.6) with soaked 70% Ethanol until glass clean. Apply 95% Ethanol over the glass and immediately add 6 μL of 3 –methacryloxypropyl trimethoxy

  Silane (Bind Silane) and 100 μL of 0.5% glacial acetic acid after that directly mix and spread the solution on the glass to covalently attach the gel onto the glass plate. Let dry for 5- 10 min.

- Treat the chamber with soaked 70% Ethanol until chamber clean. Treat the larger glass plate (33.3 x 41.9 cm) with 1 mL of dimethyldichlorosilane (Repel-Silane) to assure gel release. Let dry for 5-10 min.

- The glass and chamber are set to an electrophoresis apparatus (BioRad Sequi-Gen GT Nucleic Acid Electrophoresis Cell 38 x 50cm).

- Prepare gels (6% polyacrylamide; 7 M urea) by mixing 60 mL of the urea:acrylamide solution[1] with 750 μL of 10% ammonium persulfate (APS) and 75 μL of TEMED. Immediately apply the gel solution to the assembled gel plates (0.4 mm thickness). Allow 1 - 1.30 hr. for the gel to polymerize.
- Prerun the gel at 60 W for 60 min or until the gel temperature reaches 50°C in 1 X TBE (12.11g of 1M Tris base; 2.9 g of 0.01M EDTA; 52.5g of 0.85M boric acid).
- 48 well of comb are apply in this study. Add 5 μL of denaturing buffer (100 μL of 0.1% bromophenol blue [w/v]; 10 μL of 20 mM EDTA; 4.8 ml of 95% formamide and add DI water which is already autoclave up to 5mL) with 4 μL PCR product and 1 μL of internal marker which is depend on lower size of any microsatellite loci in each channel. When using different PCR reaction volumes, keep the same ratio to the denaturing buffer.
- Denature the samples for 5 min at 94°C in the PCR machine. Immediately place the samples on ice.
- Load samples (8 μL) to the lane as quickly as possible. Run the gel at 50 W for 90-160 min depending on the size of each microsatellite locus at 50-55°C.

Silver staining

All chemicals used for staining were analytical grade and prepared using Deionize water. The gel plates were gently shake in a shaker throughout the staining processes.

- Disassemble gel apparatus carefully, separating the glass plates. Place glass plate with gel onto a plastic tray.
- Apply 1500 mL fixing solution (10% acetic acid) and shake gently for 20 - 30 min.

- Wash gel with reverse osmosis water (RO) for 3 min, twice.
- Impregnate gel with 1500 mL of 0.1% $AgNO_3$ solution (1.5 g of $AgNO_3$ in 1.5L of DI) for 30 - 60 min, shake gently.
- Develop gel by applying, initially 1500 mL of cold (ca. 8-10°C) developing solution (45 g of Na_2CO_3 in 1500 mL DI water; 1500μL of 38% formaldehyde and 1-2 small pieces of sodium thiosulfate). Remove the glass from the $AgNO_3$ solution tray to an empty tray. Replace the developing solution immediately and gently shake until the bands appear with desirable intensity (ca. 5 min).
- Remove the glass to 1500 mL of 10% acetic acid for 10 min for stopping the developing reaction.
- Wash gel in water and air dry.

Note:

[1]**Urea:acrylamide solution**

Prepare a urea:acrylamide solution with 37.5 mL of Acrylamide/Bisacrylamide (19:1, 40%), 50 mL 5 X TBE (60.57 g of 1M Tris base; 1.45 g of 0.01M EDTA; 26.25 g of 0.85M boric acid), and 105 g. urea. Add deionize Water (DI) to a final volume of 250 mL, filter with a filtration paper, and stock at 4°C in a brown bottle.

APPENDIX H

COMPARISON OF F_{ST} VALUES BEFORE AND AFFTRE CORRECTING FOR NULL ALLELES

FreeNA is a useful program for revealing corrected F_{ST} value from microsatellite dataset that was disturbed by null alleles. The ENA correction method that performs in this program was used to correct for F_{ST} estimation in presence of null allele (see exhaustive detail in "F_{ST} Refined Estimation by Excluding Null Alleles" from FreeNA program package).

The comparison between F_{ST} values of the original data those after the ENA correction method revealed 30.5 % - 33.3% difference. Most of the F_{ST} valves from the correction for null alleles are slightly lower than the original data. The presence of null alleles in these microsatellite loci may not lead to an overestimation of F_{ST} as mentioned in Chapuis and Estoup (2007).

Statistical p-value should be calculated for the data after employing the ENA correction method. It is appropriate to indicate the significance level of population differentiation with the corrected data. However, test for pairwise corrected F_{ST} was limited in this program. Another possible approach to avoid inaccurate population differentiation that is affected by the presence of null alleles is to eliminate any locus that showed a sign of null alleles.

Slightly difference of F_{ST} between corrected and uncorrected null alleles was the reason why in this study none of the loci was removed from the test. Null alleles may not much affect the population differentiation examination.

Table 15 F_{ST} of the original data (below diagonal) and F_{ST} after the ENA correction method (upper diagonal).

	NA1	NA2	NA3	SA1	SA2	SA3	GT1	GT2	GT3
NA1	-	0.001	-0.001	0.004	-0.001	0.002	0.035	0.029	0.027
NA2	0.001 (0.457)	-	0.005	0.011	0.004	0.003	0.040	0.033	0.031
NA3	-0.001 (0.670)	0.005 (0.128)	-	0.005	0.001	0.001	0.037	0.031	0.029
SA1	0.004 (0.052)	0.011 (0.002)	0.005 (0.005)	-	0.008	0.009	0.035	0.030	0.027
SA2	-0.001 (0.268)	0.003 (0.233)	0.001 (0.117)	0.008** (0.000)	-	0.000	0.037	0.034	0.030
SA3	0.002 (o.444)	0.003 (0.508)	0.001 (0.218)	0.009** (0.000)	0.001 (0.401)	-	0.033	0.026	0.022
GT1	0.035** (0.000)	0.040** (0.000)	0.037** (0.000)	0.036** (0.000)	0.037** (0.000)	0.034** (0.000)	-	-0.002	0.001
GT2	0.030** (0.000)	0.034** (0.000)	0.031** (0.000)	0.031** (0.000)	0.035** (0.000)	0.027** (0.000)	-0.002 (0.900)	-	-0.002
GT3	0.027** (0.000)	0.032** (0.000)	0.029** (0.000)	0.027** (0.000)	0.030** (0.000)	0.022** (0.000)	0.002 (0.666)	-0.002 (0.844)	-

Note: Values underlined indicate difference of F_{ST} between uncorrected and corrected null alleles.

APPENDIX I

POWSIM; TO DETECT STATISTICAL POWER OF THE DATA SET TO DETECT POPULATION DIFFERENTIATION.

Fisher's exact test is an important statistic for examining genetic homogeneity between populations. Therefore, the statistical power of the test to reveal true difference - i.e. the probability of rejecting the null hypothesis (Ho) when it is false – should not be bias from any reason such as the number of samples, sample sizes, number of loci and alleles, allele frequencies, and degree of differentiation (quantified as F_{ST}). POWSIM becomes a more popular program for estimating power of Fisher's exact tests and the statistical α (type I) error when evaluating the hypothesis of genetic homogeneity using the empirical data set.

How to perform POWSIM

1. Program is available as a free download for every user. Setting program follow suggested step.

2. Input file was required in text file. Each line has detail as below.

Line 1 is Optional descriptive text:

Population of *Lutjanus russelli* in Thailand

Line 2 Optional descriptive Text:

9 microsatellite loci with 31 16 30 36 28 43 19 45 and 27 alleles, respectively

Line 3 Three integers defining parameters (the number of dememorizations (burn in), batches, and iterations per batch) used in the Markov chain process underlying Fisher's exact test:

1000 100 1000 Iteration/permutation factors (Fisher's exact test) [1]

Line 4 Two "switches" (1=yes, 0=no) instructing POWSIM to estimate power for either or both of the chi-square and Fisher testing approaches:

1 1 Run chi-square and/or Fisher's exact test (1/0)

Line 5 A switch (1=yes, 0=no) telling POWSIM whether or not to delete detailed output files:

0 Erase detailed output (1/0)

Line 6 Blank line (cannot be excluded).

Line 7 Number of polymorphic loci included in the analysis:

9 Loci

Line 8 Number of subpopulations sampled:

3 Populations (s)

Line 9 Effective size of subpopulations (*Ne*)[2]:

5000 Effective size when drifting apart (*Ne*)

Line 10 Generations of drift before sampling (t)[3]:

10 Generations of drift (t)

Line 11 The number of (diploid) individuals sampled from each of the subpopulations in generation. The number of entries must be the same as the number of subpopulations.:

106 117 77 Sample sizes (when sampling after completed drift process)

Line 12 The number of times (runs or replicates) to repeat the entire process of drift, sampling, and statistical testing.:

1000 Number of simulation runs/replications

Line 13 Blank line (cannot be excluded).

Line 14: Header line for allele frequencies (cannot be excluded).:

Number of alleles and allele frequencies for each locus in base population:

Line 15 - 23: The number of alleles (integer) and their frequencies (real) at locus 1 - 9.

Note: [1] is default number of parameters set

[2] Selection Effective sizes (Ne) for examine statistic power of test should be maintained overall allele frequencies or similar to those of base population to prevent excessive loss of low frequency alleles. In this case *Ne* was varied between small (100) to large (10000) because large *Ne* would be expected to maintain more alleles, resulting in a better representation of the intended allelic frequency distributions, however small effective sizes should not be ignore as well.

[3] Generations of drift before sampling (t) was obtained from the combination between F_{ST} and *Ne* suggested in user's manual of this program. F_{ST} was selected cover the empirical F_{ST} in this study, ranging from 0.001 to 0.037. Therefore, the F_{ST} value was set at 0.001, 0.005, 0.02, 0.05 and also F_{ST} = 0 for revealing the type I error that is obtained by omitting the drift step. Generations of drift (t) was varied depending on the suggested *Ne* and F_{ST} combination.

3. The statistical power of the data set was obtained by multiplying 100 with Fisher value at proportion of significances ($P<0.05$) for summed/combined test statistics.

Statistical power of the data set in this study

The statistical α (type I) error that was obtained by omitting the drift step (F_{ST} =0) showed a low value of 4.7%-6.3%, implying that the probability of rejecting Ho when it is true is low.

Moreover, POWSIM showed a high statistical power of the data set to detect significant population differentiation among 3 population groups (The GT, the NA, and the SA). Both small and large effective size (*Ne*) in various generations of drift do not affect the statistical power of the data set (83.1%- 85% for low F_{ST} (F_{ST} = 0.001), and 100% for highFst (*Fst* = 0.05)) (Figure 23). Therefore, the number of samples (over 70 samples for each location), number of loci (9 loci) employed in this study are enough for obtaining a high statistical power to test for population differentiation. Increase the number of samples or the number of loci was not necessary.

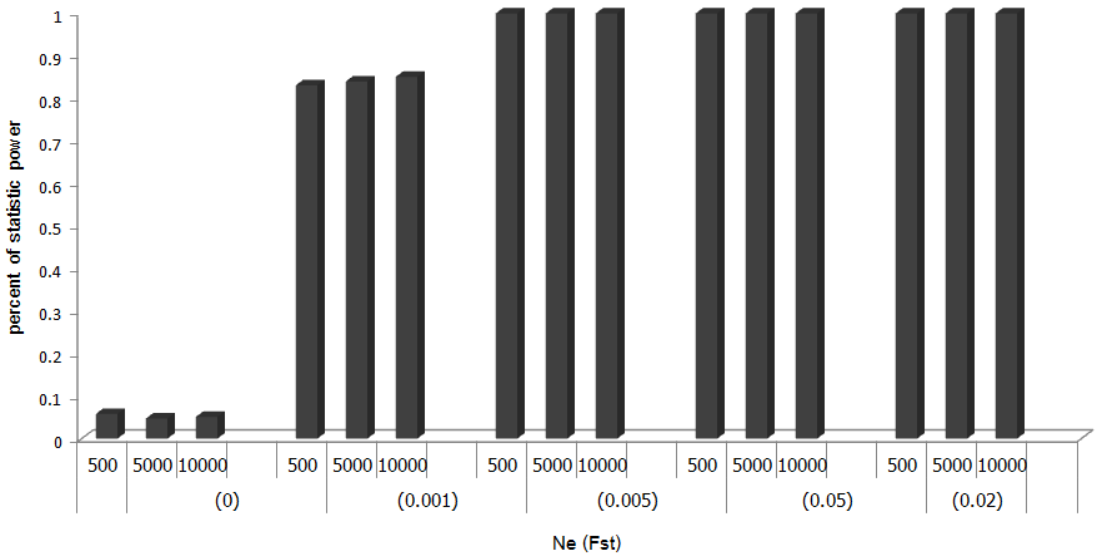

Figure 23 Percentage of statistical power of the data set at different sample sizes and F_{ST}

APPENDIX J

DETECTING THE NUMBER OF CLUSTERS

Assigning individuals to their populations of origin is the basis step for population genetic test that examines allele frequencies among and between populations. The appropriate software for detecting the cluster number is STRUCTURE, based on Bayesian algorithm. Most studies are based on simulations with limited number of populations around two to four populations (Pritchard *et al*., 2000) or an absence of dispersal between them. Few studies mentioned about the performance of STRUCTURE in case of samples have more intense dispersal among groups or unclear complex dispersal patterns between them. Recently, ΔK is used to identify the most possible K based on the second order rate of change with respect to K of the likelihood function created by STRUCTURE.

How to detect most possible number of populations (ΔK)

1. Plotted the mean value of ln likelihood (L(K)) over 10 runs (Figure 24), L(K) obtain from final results by STRUCTURE.

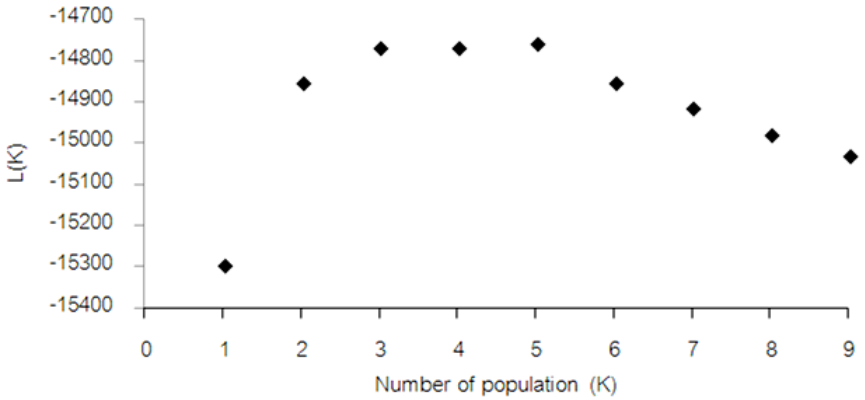

Figure 24 Relative between the mean likelihood L(K) over 9 hypothetical K

2. Plotted the mean difference between successive likelihood values of K, that is L´ (K) (Fig. 25).

$$L´(K) = L(K) - L(K-1)$$

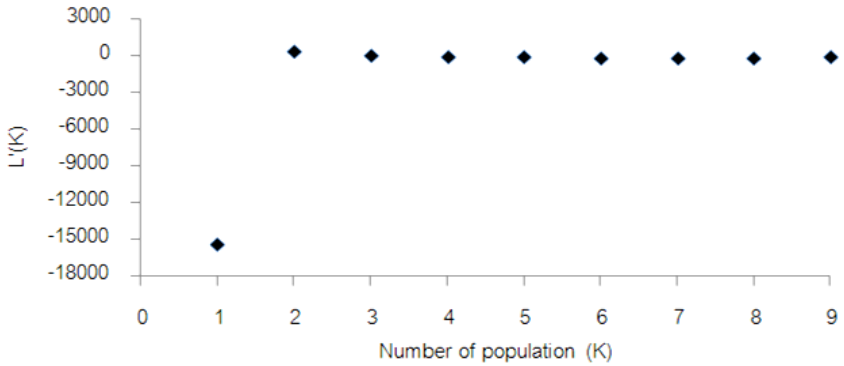

Figure 25 Relative of the mean difference between successive likelihood values L'(K) over 9 hypothetical K

3. Plotted the (absolute value of the) difference between successive values of L´(K), that is L˝(K) (Figure 26).

$$|L˝(K)| = |L´(K + 1) - L´(K)|$$

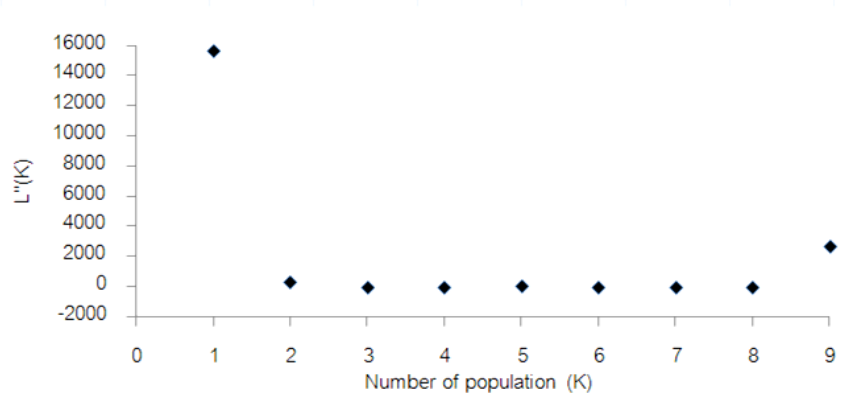

Figure 26 Relative of the mean difference between successive of L"(K) over 9 hypothetical K

4. Estimated ΔK as the mean of the absolute values of L″(K) averaged over 10 runs divided by the standard deviation (s) of L(K) that obtain from final result by software. Plotted ΔK and number of expected K that we put in the software (Figure 27).

$$\Delta K = m(|L''(K)|)/s[L(K)],$$

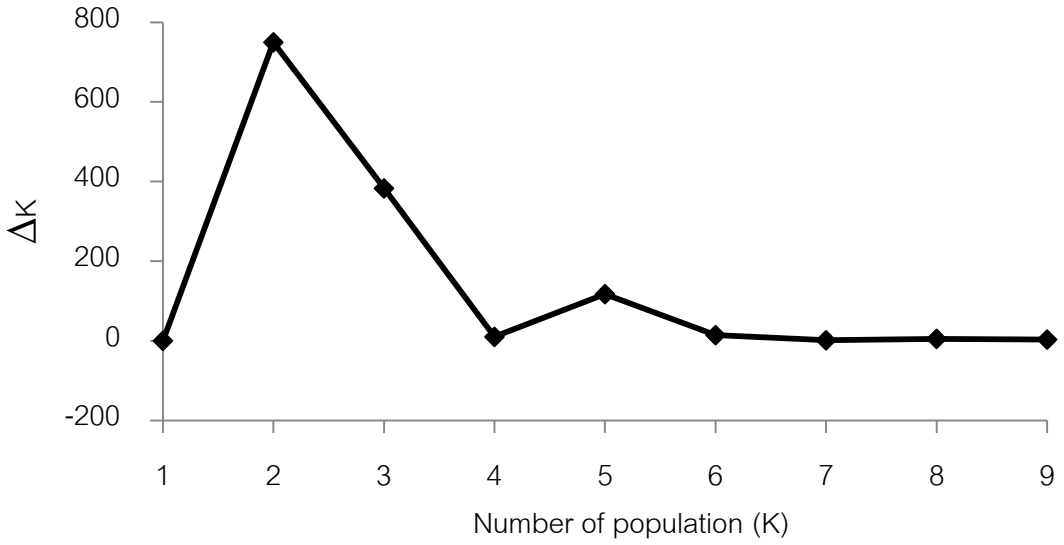

Figure 27 Relative of ΔK over 9 hypothetical K

How many population (most possible K) in this region

In most cases, the mean of likelihood increased until the most possible K was reached. STRUCTURE revealed 3 possible populations this study (Figure 24), whereas ΔK suggested only 2 possible populations (Figure 27). In general, STRUCTURE does not have a clear or disreputable performance when clusters of samples have more intense dispersal among groups. Log likelihood which reported from program may be predicted by allowing supernumerary finding the hidden group within population. It would show uppermost of clusters, but does not provide a correct estimation of the number of clusters. However, ΔK was used to help identifying the most possible K. Using ΔK, it was found that

there are two populations of *L. russelli* in Thailand, which are the GT and the Andaman Sea populations.