

Supplementary Information

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Table S1. Transcriptome quality trimming, assembly, and microsatellite loci detection statistics for the five *Hynobius* species in Taiwan.

	<i>H. fucus</i>	<i>H. sonani</i>	<i>H. glacialis</i>	<i>H. formosanus</i>	<i>H. arisanensis</i>
Total raw reads	102,388,724	98,761,318	83,079,509	100,638,542	43,030,802
Total reads after QT*	102,318,304	98,661,924	82,969,159	100,536,634	42,857,826
Average length	137.36	137.28	137.40	137.27	137.30
Total bases after QT*	14,054,672,800	13,544,435,014	11,403,148,921	13,800,527,963	5,885,723,709
Total number of transcripts	170,727	209,217	217,222	240,877	119,546
Total number of identified SSRs	43,035	54,571	54,808	61,019	35,557

*QT = quality trimming (criteria: min length = 35 bp, error probability < 0.05).

Table S2. Transferability of the 133 candidate cross-species EST-SSR markers.

Locus name	Primer sequence (5' > 3')		Repeat unit	No. of repeat units	Expected PCR product length	Transferability
	forward	reverse				
Locus01	AGGTGGCTGTGATAGGCAAG	TCAAGGTCGTGAACGGTGTA	TCT	10	233	Y
Locus02	CTGCTCAGACAGAAGTTGTCTG	TGGTATTAGGATGGAAGAGG	ATCT	12	250	N
Locus03	GCGGTGCAGAATTATATGTCC	GTTCAAGACCAGCAGACAGG	TCA	12	168	N
Locus04	GTTTCGCTCTCGAACTTCAGG	CGCAATAGCGAGTGAGTGAG	CTCA	10	210	Y
Locus05	TGTCTCAGTCTGTTGGTTACGG	AAGGTGTGTTCTTTGTGCTG	ACTC	9	229	Y
Locus06	ACAGCGCCACTGAGGATTAC	CCTCCAGCTGTAATCGGATG	CAC	10	225	N
Locus07	TTGACATCAAACAGGGTATTC	GCAGTGTGAAAGAAGGCAGAG	GATA	9	153	Y
Locus08	GAGGAAGAAGAATGCAGTGAGG	TCTGTAATATCTTGCATCTCC	GAG	10	215	Y
Locus09	GTGAGACCTTCAGCCAGACC	CCGTGCTCAATAAGATAAGG	CTAT	12	224	N
Locus10	TCAGGTGGGTGGTAAAGGTC	AATGGCGATAACAGTTCACCT	TTC	11	203	Y
Locus11	TTGTACTTGCCATCTGAGC	TGGAGCTCTATCTACTAAGACC	TCT	20	216	Y
Locus12	GGCTCTGCAGTGTGATCG	AACGCCTGCTGAAGTTCG	CAG	10	220	N
Locus13	ACACTCCGGCAATACACACC	TCGTGCACTGACTGAACAGG	ATAC	16	296	Y
Locus14	CTTCAGCCAGCCAACAGC	CACCAGGAGAAGCAGAGACC	CAG	14	195	Y
Locus15	CCCAGTGAAGTTTGTGTGC	AAAGCTTCCTGTGTACGTTGC	ATT	15	212	Y
Locus16	GGTGCCACACACATACAAGG	GCTCGTCATCGCTTCTGC	CAC	6	220	N
Locus17	CACCGAAGTGTCAACAGC	CGTCAAGCAGCAAGTACAGG	ATG	9	236	N
Locus18	TCAGAACATCCAGACGAAGC	AGCGGACGTATCTGATTGG	TGA	9	189	Y
Locus19	TGGGAGGAGCTGTGAGTAGG	TGTACAGCCTGTGCCTCTCC	AGA	7	188	Y
Locus20	CGATTAGTTCGCAATTGTAGC	GTCAGTTGCCATGACTGTGG	ATA	8	245	N
Locus21	ACAACAGCAGCAGCAACG	GGTCCATACGTGCCATCC	CAG	14	177	N
Locus22	GCGGTGCAGAATTATATGTCC	GTTCAAGACCAGCAGACAGG	TCA	12	168	N
Locus23	CACTCTTGCTCCAGCATCC	GCAATGAGCAGACAACATGC	ATA	7	288	N
Locus24	GAGTGTGTGAGCTTGCTTGC	AGGTCAGACTGAAGTCCAGAG G	TTA	8	240	N
Locus25	GGAAGAGCAGTCTGATGACG	TCTTCCTGGACCATCTCTGG	GGA	8	389	N
Locus26	AAGACAGCGCTATATAAGACTTGC	AGTCTGGCATCAACGCTCTCC	ATT	7	250	Y
Locus27	TGTAATTGGCGCTATATAAATGTA AAC	AGGTTCTCTGTTGGTCATTG	ATT	8	237	Y
Locus28	AAGAGATGCGTCATCCTTGG	ATCACGGCCTCCCTCTCTAC	AAT	7	211	Y
Locus29	TGAGTCTGGGTAAGACTGCAC	CTGTGCTCATGTACGGCATC	ATT	7	152	N
Locus30	CCCCAAATCTGATATTATTATTGG TAA	GAGCAGTAGTGGCTGGAAC	TCT	7	232	Y

Locus name	Primer sequence (5' > 3')		Repeat unit	No. of repeat units	Expected PCR product length	Transferability
	forward	reverse				
Locus31	GAGAGACACTCTTGACCCAC	CCAACCAGGATATTCCTTCC	TCTTT CC	8	286	Y
Locus32	AAGAGAACGCAAGAGATCACC	GAGGAGGATGATGAGGAGTAG G	TCTTT CC	8	578	N
Locus33	GAGAGACACTCTTGACCCAC	CCAACCAGGATATTCCTTCC	TCTTT CC	8	286	Y
Locus34	CACCGAAGTGTGACAACAGC	CGTCAAGCAGCAAGTACAGG	ATG	9	236	N
Locus35	GCTAGGTATGCGCTTAGACG	CGCTACCACCAATTCTAGCC	TTTC	6	646	Y
Locus36	TGGGAGGAGCTGTGAGTAGG	CCTCTCCGCCTGTGTGCC	AGA	7	175	Y
Locus37	TGGGAGGAGCTGTGAGTAGG	CCTCTCCGCCTGTGTGCC	AGA	7	175	Y
Locus38	CCTAGAGTCGCAATGTGTGG	GAGAGGGCACCATTATCTGG	ATT	7	155	N
Locus39	GCCTGGATGAGAAGTTTAGGG	GAGTAAGGGACCGGTTTATGC	AGC	6	214	Y
Locus40	CCTACATGGCAGCTTATCAGG	TACGTGCTGGATATGAACG	CAC	5	222	Y
Locus41	CCAGCAATATAGCCGAACG	GCCGCTTATCTTCCATCC	GAG	8	242	N
Locus42	GATGCAAGATGCTGGAAGC	TATTGTTGCACGTCGTGAGG	ATT	7	150	Y
Locus43	TTGAGAGAATCGCTCCGAAC	CGTGTGCAGGTCGTAGTCTG	CAG	7	250	N
Locus44	CTCGGGAACAAGTCTGTGG	CAGTGGCTAGGCAAGATGG	ATGT T	8	223	Y
Locus45	GAACCACCACTCGTTCTGC	CGCAGCAATCTGTTATCACC	AAT	7	236	Y
Locus46	ATGGAGCAGGAAGCAAGAAG	CTCCGTGTGAGGTCTTGGAC	ATA	7	239	Y
Locus47	CTCCTGTTGGTTGGACTGG	GGTGGAGGAGCTACTTGTCTG	CCA	7	218	Y
Locus48	CTCTCCGTATGCAGTCAGACC	CGTCTGAAGACCTGGAAGC	GCA	9	183	Y
Locus49	CAGAATGAAGATGGCACAGC	TCCCTCGTGGTAGCATGG	GCA	9	170	Y
Locus50	AGTTCGCTAGCTCGCTTCC	AAGGATACCATACTAAGAGCA TCG	ATA	8	247	Y
Locus51	AGCCTTCATCCCGATTAC	CGTTCATCACGCTCTTCTTG	ACC	5-6	187	N
Locus52	GGCAGGTGGCAACAAAGG	CGTCACTCTCATTGGCTTCC	GTG	5	245	Y
Locus53	CCACTACAGCAGCATGTC	AAACCGTCTGTTCCGAGTC	GGA	3-6	210	N
Locus54	TGGTGATGAGGAGGAAGAGG	GAACCACCCGCTTTGTACTG	ATG	5-7	177	Y
Locus55	TCTGAGATTCTTCACTGCTTGG	AAGGCAATATTGAGTGTGAAG G	ATT	5	228	Y
Locus56	GCGCCTTGCAGTATGAGC	CCATGTCGGTCTCACTTGC	GAG	5	163	N
Locus57	GACAGCGGCTTCCAGGAG	ACATCGATTGTTGCTCTCCA	GGC	3-5	195	Y
Locus58	TGAAAAGGAAGCCAGTGAGG	CTCTCATCGTCGCTGCTACC	AGG	5-6	198	Y
Locus59	AGGTTTTACCCCTTTCTCC	CATGGGAAGTGTATCAGTGG	TGA	3-8	205	N
Locus60	GTGCCACAACTTGGAACCC	TTATTAAGCGGGCCAAACAC	GTG	4-5	181	Y
Locus61	CTGATGATGAGGAGGAAGAGG	GCATATGACACCACCGTTCC	GTC	5	198	Y

Locus name	Primer sequence (5' > 3')		Repeat unit	No. of repeat units	Expected PCR product length	Transferability
	forward	reverse				
Locus62	TTTGCTGGATCTGCTTGTGTG	ACTACTGCCACCTCCACCAC	AGC	4-6	247	N
Locus63	GCGCCTTGCAGTATGAGC	CCATGTGCGGTCTCACTTGC	GAG	5	163	Y
Locus64	CGGCGGATCCCTCTCTCC	GATGCAGGGCAATACTCAGG	GCT	5-7	208	N
Locus65	CGGACTGCGAGGTGATCC	TCGGCGACGCATCTATGG	GAG	5-7	174	N
Locus66	CTCAAATGAACCTGCGTTGG	ACACAAAGGACGGACAATCC	GAA	5	236	N
Locus67	GAGTCCTTCAGGCCACACC	CTGCTGGGAGGATGTAGACG	CAC	4-5	198	Y
Locus68	GAAGTAGAAGGCCGTGTTC	CGCTGCGGAGGTACGACA	GCA	4-5	109	Y
Locus69	CCATTCCAGCGAACAAAGC	GGTCATCATGGCTCACTGC	GCT	5	212	N
Locus70	ACCACCTGCATCGACACC	GCTGCCGTCGTTGTTAGC	CAC	3	192	Y
Locus71	AGCCGCCAAGCAGGAAGG	TTTTCTGTTTAGGGCACTGG	AGC	4-5	-	N
Locus72	AAGAGGTGTGTCATACATGG	GGTAATGCGTACACTGCATAG G	TGT	5-6	189	Y
Locus73	CAAGCTCTTTGGCATCAGC	TTGATGCGGTGTAGTTGTCC	GGA	4-5	158	Y
Locus74	CTCTCCGTATGCAGTCAGACC	CCGTCTGAAGACCTGGAAGC	CCA	5	187	Y
Locus75	TGGGTAATCCGCCACTGC	GCTGACCCAGGAGGTTGC	GCA	8-9	184	Y
Locus76	TACAACATTCTGACCAGGAG	CGGCTTCTCATCACCTGAG	GAG	4-5	287	Y
Locus77	CAGTTTCCTCCTCTGTGG	GGTGGAGGAGCTACTTGTGC	CCA	6-7	228	N
Locus78	GAACTGCAAGGGAAGAGTGC	GCAGCATCTCCAGTGCTCC	GGA	5	241	Y
Locus79	TTGCAGTTGCATGCTTTAGTG	CCGTCTGGTCTTCATTAGCAG	TGGA	3-5	232	Y
Locus80	CGAGGAAGTCAGTCACATGG	GGAGTGACCTCTGACATCTGC	CAC	4-5	198	N
Locus81	CCAGCAATATAGCCGAACG	GCCGCTTATTCTTCCATCC	GAG	4-8	242	N
Locus82	ATCCTCAGAGGCTCGGTAGC	AAGCTTCGCTAGCACAATCC	TGA	3	246	N
Locus83	GGTCCACTCAGCCTATCATCC	TGAAATACAGCCCGTTAGAGC	ATT	3	177	Y
Locus84	TCCGAATCTGAGGATGAAGG	TTGTGGATCATGGACTAAATGG	AAG	5	183	Y
Locus85	GAGGCTCTTCATTATTGTGC	GTCTGAGAGCTCAGCCATGC	CAG	2	232	Y
Locus86	GAATTTGGAGAGGACGGAAC	TCTCCCTCCCAAACACAATG	GAT	5	214	Y
Locus87	CTGCCACAGCACTTAGATTACC	TGACAACATCGTATCGGAAGG	GAT	4-7	228	Y
Locus88	TCTTATGGTCCTGGGATTGC	TGCACTGATAGAGATGGATGC	TCAA	6	199	Y
Locus89	GGAGCAAATAAACAGCACACC	AACAGACGTGGGATACATAGG	CACT	4-5	151	Y
Locus90	GGACACGTGAGAGATCAGAGG	GGGTTTCATGAGGGTAAATTCC	GAG	3	200	N
Locus91	CCCTCCCCCTCATATTTC	GAGCTACTTGGCATCACTTGC	GTG	5	210	Y
Locus92	TGAAGGAGGAACTTTGGTG	ATCCTGCCAACTTCCACTTG	GGT	4-5	160	N

Locus name	Primer sequence (5' > 3')		Repeat unit	No. of repeat units	Expected PCR product length	Transferability
	forward	reverse				
Locus93	AACGGCAACAGCGAGAGC	GCAACAGCTGAACCACAAGC	GAG	5	391	N
Locus94	CCGGTTCTCCTGTAGTTGC	GACCGCGCTATACAAAGTCC	GTTG A	4	202	Y
Locus95	CCAGGTCGAGGAAGTCAGTC	TGGAATCCATGGTCTCAAGG	CAC	4-5	206	N
Locus96	ACCGTCTCAGCAGCTAAACC	TTTGTGCTCCTCTGAATGG	GCA	6-8	226	Y
Locus97	ATCTTCATGGGCGAGAAGG	CTGTCAGTAGGTGGGATCTCG	AGA	5	203	N
Locus98	TGAAGGAGGAAACTTTGGTG	TTCAAATCCTGCCAACTTCC	GGT	4	165	Y
Locus99	CTGAGCGCAGCATGGAGAG	GCTCACATCCGTCCAGTAGA	GCT	5-6	227	N
Locus100	TGGCTGAAGACCAGAGTAAGC	GGACATCTTCAGCAGTCTGG	GAG	5	219	Y
Locus101	AGTACTGTACACGCCAACG	GTAGGTCTTGGAGGCACAGC	CCA	4-5	-	N
Locus102	CAGAAGCAATTGCCAATCC	AGAGGCAGCTTAGGCTGTGG	TGAG	5	161	N
Locus103	CCGTAATTCACGCTTCAACC	ACTCAGCCTTCCATCCTTCC	TTGA G	5	197	Y
Locus104	TGAGCCCAGAGGGTATAAAGC	TGGTGACCTAAGTGCTGTGC	AC	6	215	Y
Locus105	AGACTCGGAGCCCTAAATGC	TATTGCGAATATGGCCAAGC	AT	6	203	Y
Locus106	TGTTTGAGGAAAGTGAAAGG	ACTCCCTGTAGAGGTTACGG	TA	6	241	Y
Locus107	TGGTCACCTGCTATTCAGAGG	CTGATCCTCTGACCACTCG	AT	17	191	Y
Locus108	TGTCCAACCTGCAGACTCC	GGCAAGCCTACACCTGTGC	GT	7	223	Y
Locus109	GGATGGTCGCAGAATGATGG	CGTGATCGCCATTGACAGC	GT	11	151	Y
Locus110	GCGAGGGCTGTAATTGTGC	CGGTTGTGTGCCATGTCC	AT	6	248	Y
Locus111	GCTCACTCAGATGCCTGTCC	TCCACAGCAGCGATTAAGG	TG	9	173	Y
Locus112	GGACAGAGGGCAATACAC	CTGAGTCCCTGGCTTCTC	AG	12	162	Y
Locus113	CGGAACGTTACTCCCAGAAG	CCTCCAGCTCACAGGAGTTC	AT	6	225	Y
Locus114	GCTCCATAACGGTTCCTTAG	CGATCACAAATCCCAAAGAC	AT	6	225	Y
Locus115	AATTAACATGCTTGTGTATAGC	AGGTTATCCTTCTGATGTCC	AT	7	199	Y
Locus116	GAATCTCGTCTTTGCTGTGG	TGTGGCATTAGAAAGTAAAG C	GA	7	174	Y
Locus117	TGAACATGAATGGTACAGTCC	GGCTTTGGACATTCTATTAAGC	GT	6	248	Y
Locus118	AAGCTCGCAGAGGATCTCG	CATTTGCTATGCCGAAGTACC	AT	10	183	Y
Locus119	ACACAGTCACCTGCCATACG	CCTAGTCTGGGAAGCTGATGG	TC	6	247	Y
Locus120	AACCTTATTGGTTTCCTTCCAG	TTCCTGCACATCCACAAATC	GT	6	187	Y
Locus121	TCTGCACGTGTCTGTCTGTG	AGCAGCTGTGGCCTTAACAC	AC	11	180	Y
Locus122	AGGAAGCACACGCGAGAC	CTCAGCTGGAAGCTCGTTG	TC	6	247	N
Locus123	CTTAAATCGCTTGCATGACC	AGCTATGTCAACACGCAACC	AT	7	228	Y

Locus name	Primer sequence (5' > 3')		Repeat unit	No. of repeat units	Expected PCR product length	Transferability
	forward	reverse				
Locus124	CCTAGATGTCTCCGTTCTTCC	CAGCCAAGATGAAGTGAATCC	AT	6	240	Y
Locus125	ATGATGCGCTTCTACATCC	TGTTGACCAAAGCAGATAGC	TC	6	220	Y
Locus126	GGAACAATGTCTGTCATGC	ACGTATGGGTGTGTACGG	CA	9	154	Y
Locus127	CCAGGTTCTATCATTGCTC	CAGAAGGGCTACAAGGTG	AT	8	207	Y
Locus128	TCGATATGGAGACTGAAACC	TCGATATGGAGACTGAAACC	CA	9	174	N
Locus129	CTGAAACAAATTACCCTTGC	GATTGTGTCAGAGAGATCAGG	TA	16	177	Y
Locus130	TTGCGAGTTCATTACTAAAGG	TCTAATGCGTCAATTATGTCC	AT	6	237	Y
Locus131	ACCAAAGGACATCGTTCC	AAGACAGAGACAGCCAACC	CT	6	183	Y
Locus132	ATTCATGCACAGAGTGTCTAC	CGTTCTCTGCACTACTGAGG	CA	20	247	Y
Locus133	TATGCATGCTGCACTTCC	AATACCGTGGAACATAGTGC	AT	9	214	Y

Table S3. Taxonomic assignment of Taiwanese salamanders based on mitochondrial cytb haplotype and morphotype.

Taxonomic assignment		Haplotype (N)
cytb haplotype	morphotype	
<i>H. fucus</i>	<i>H. fucus</i>	HF01(1), HF02(1), HF03(9), HF04(1), HF05(2), HF06(1), HF07(1), HF08(3), HF09(1), HF10(1), HF11(1), HF12(3), HF13(1), HF14(1), HF15(1), HF16(1), HF17(1)
<i>H. sonani</i>	<i>H. sonani</i>	HS01(1), HS02(3), HS03(1), HS04(1), HS05(3), HS06(1), HS07(2), HS08(1), HS09(2), HS10(2), HS11(3), HS12(2), HS13(1), HS14(1), HS15(1), HS16(1), HS17(1), HS18(1), HS19(2)
<i>H. glacialis</i>	<i>H. glacialis</i>	HG01(7), HG02(6), HG03(5), HG04(1), HG05(1)
<i>H. formosanus</i>	<i>H. formosanus</i>	HT01(1), HT02(1), HT03(4), HT04(1), HT05(1), HT06(1), HT07(1), HT08(1), HT09(1), HT10(1), HT11(1), HT12(1), HT13(1), HT14(1), HT15(2), HT16(2), HT17(1), HT18(1), HT19(1), HT20(1), HT21(1), HT22(2), HT23(2)
<i>H. arisanensis</i>	<i>H. arisanensis</i>	HA01(1), HA02(1), HA03(2), HA04(1), HA05(1), HA06(1), HA07(1), HA08(2), HA09(1), HA10(2), HA11(1), HA12(1), HA13(1), HA14(1), HA15(1), HA16(1), HA17(1), HA18(2), HA19(1), HA20(2), HA21(1), HA22(1), HA23(1), HA24(1), HA25(1)

Table S4. Genetic variation and polymorphism of 20 EST-SSR loci in *Hynobius fucus*.

Locus	N	k	Ho	He	PIC	LD	HW	AR
Locus13	30	3	0.310	0.630	0.538	5	**	3.267
Locus26	30	4	0.633	0.539	0.421	1	NS	1.000
Locus30	30	3	0.133	0.513	0.407	3	**	6.135
Locus52	30	2	0.400	0.427	0.332	0	NS	3.000
Locus58	30	4	0.233	0.315	0.279	0	NS	4.980
Locus60	30	1	0.000	0.000	0.000	0	**	2.000
Locus76	30	7	0.400	0.669	0.624	2	**	3.975
Locus79	30	5	0.233	0.740	0.684	6	**	2.000
Locus87	30	2	0.167	0.259	0.222	0	NS	3.502
Locus88	30	4	0.100	0.401	0.372	3	**	3.267
Locus89	30	2	0.200	0.398	0.315	5	*	1.955
Locus91	30	4	0.233	0.295	0.270	3	NS	2.869
Locus94	30	2	0.100	0.097	0.090	0	NS	2.000
Locus96	30	2	0.267	0.472	0.357	0	*	2.000
Locus104	30	2	0.103	0.216	0.190	0	*	5.237
Locus105	30	6	0.133	0.491	0.456	5	**	2.000
Locus108	30	1	0.000	0.000	0.000	0	**	15.108
Locus114	30	17	0.833	0.922	0.900	4	NS	2.984
Locus123	30	5	0.414	0.528	0.474	1	NS	1.000
Locus131	30	3	0.133	0.296	0.271	0	**	4.537
mean	30	3.95	0.251	0.410	0.360	1.900	-	3.641

N: sample number; k: allele number; Ho: observed heterozygosity; He: expected heterozygosity; AR: allele richness; HW: *p<0.05, **p<0.01, NS; LD: linkage disequilibrium, representing the number of linked loci. PIC: polymorphic information content.

Table S5. Genetic variation and polymorphism of 20 EST-SSR loci in *Hynobius sonani*.

Locus	N	k	Ho	He	PIC	LD	HW	AR
Locus13	30	6	0.333	0.618	0.535	0	**	12.366
Locus26	30	6	0.500	0.748	0.692	5	**	4.252
Locus30	30	4	0.100	0.160	0.153	0	*	4.953
Locus52	30	3	0.300	0.406	0.356	5	NS	3.868
Locus58	30	13	0.533	0.891	0.864	0	**	5.483
Locus60	30	6	0.192	0.474	0.435	5	**	2.000
Locus76	30	5	0.200	0.761	0.713	0	**	5.403
Locus79	30	7	0.533	0.784	0.737	1	**	2.633
Locus87	30	2	0.300	0.503	0.372	0	*	7.483
Locus88	30	6	0.367	0.688	0.619	6	**	4.633
Locus89	30	4	0.133	0.342	0.305	3	**	3.824
Locus91	30	9	0.600	0.841	0.806	2	**	4.006
Locus94	30	4	0.233	0.408	0.360	1	**	2.995
Locus96	30	4	0.267	0.613	0.541	1	**	4.900
Locus104	30	4	0.200	0.689	0.618	0	**	6.242
Locus105	30	5	0.167	0.675	0.608	6	**	4.627
Locus108	30	4	0.167	0.693	0.625	0	**	13.404
Locus114	30	15	0.464	0.842	0.814	2	**	2.000
Locus123	30	8	0.367	0.829	0.790	3	**	4.955
Locus131	30	3	0.233	0.310	0.268	0	NS	6.724
mean	30	5.900	0.309	0.614	0.561	2.000	-	5.34

N: sample number; k: allele number; Ho: observed heterozygosity; He: expected heterozygosity; AR: allele richness; HW: *p<0.05, **p<0.01, NS; LD: linkage disequilibrium, representing the number of linked loci. PIC: polymorphic information content.

Table S6. Genetic variation and polymorphism of 20 EST-SSR loci in *Hynobius glacialis*.

Locus	N	k	Ho	He	PIC	LD	HW	AR
Locus13	20	7.000	0.400	0.684	0.613	8.000	*	9.850
Locus26	20	5.000	0.133	0.414	0.373	0.000	*	4.000
Locus30	20	3.000	0.300	0.382	0.333	5.000	NS	6.949
Locus52	20	6.000	0.379	0.814	0.771	3.000	**	4.850
Locus58	20	13.000	0.500	0.901	0.875	3.000	**	2.999
Locus60	20	6.000	0.414	0.820	0.777	5.000	**	2.999
Locus76	20	8.000	0.433	0.823	0.784	7.000	**	2.000
Locus79	20	6.000	0.500	0.714	0.650	1.000	**	3.000
Locus87	20	3.000	0.167	0.244	0.227	0.000	*	5.950
Locus88	20	3.000	0.233	0.321	0.293	3.000	*	3.900
Locus89	20	5.000	0.700	0.680	0.606	3.000	*	3.950
Locus91	20	6.000	0.533	0.679	0.629	2.000	NS	3.000
Locus94	20	5.000	0.367	0.707	0.642	5.000	**	3.999
Locus96	20	6.000	0.500	0.689	0.615	1.000	**	2.000
Locus104	20	4.000	0.233	0.521	0.457	1.000	**	3.000
Locus105	20	6.000	0.400	0.631	0.571	4.000	*	2.000
Locus108	20	4.000	0.267	0.545	0.484	0.000	**	6.847
Locus114	20	8.000	0.267	0.542	0.495	1.000	*	2.000
Locus123	20	2.000	0.033	0.210	0.185	3.000	**	3.000
Locus131	20	2.000	0.367	0.463	0.351	0.000	NS	1.000
mean	20	5.400	0.356	0.589	0.537	2.750	-	3.86

N: sample number; k: allele number; Ho: observed heterozygosity; He: expected heterozygosity; AR: allele richness; HW: *p<0.05, **p<0.01, NS; LD: linkage disequilibrium, representing the number of linked loci. PIC: polymorphic information content.

Table S7. Genetic variation and polymorphism of 20 EST-SSR loci in *Hynobius formosanus*.

Locus	N	k	Ho	He	PIC	LD	HW	AR
Locus13	30	3.000	0.067	0.242	0.221	1.000	**	10.251
Locus26	30	5.000	0.467	0.654	0.598	0.000	*	4.739
Locus30	30	2.000	0.467	0.452	0.346	3.000	NS	4.500
Locus52	30	8.000	0.667	0.814	0.772	2.000	*	2.950
Locus58	30	12.000	0.800	0.849	0.818	0.000	NS	4.955
Locus60	30	6.000	0.333	0.716	0.651	0.000	**	1.869
Locus76	30	4.000	0.333	0.372	0.340	0.000	NS	3.633
Locus79	30	6.000	0.567	0.742	0.684	2.000	**	4.724
Locus87	30	2.000	0.067	0.066	0.062	0.000	NS	6.481
Locus88	30	5.000	0.167	0.507	0.466	1.000	**	4.739
Locus89	30	5.000	0.300	0.589	0.523	0.000	**	2.950
Locus91	30	7.000	0.633	0.707	0.666	0.000	NS	2.000
Locus94	30	3.000	0.267	0.244	0.225	1.000	NS	7.136
Locus96	30	5.000	0.433	0.538	0.472	1.000	NS	4.266
Locus104	30	3.000	0.633	0.603	0.514	0.000	NS	2.955
Locus105	30	3.000	0.200	0.382	0.333	1.000	*	3.000
Locus108	30	4.000	0.600	0.596	0.524	0.000	NS	5.583
Locus114	30	5.000	0.667	0.655	0.594	0.000	NS	2.000
Locus123	30	2.000	0.133	0.183	0.164	0.000	NS	3.955
Locus131	30	4.000	0.167	0.337	0.295	0.000	*	2.000
mean	30	4.700	0.398	0.512	0.463	0.600	-	4.23

N: sample number; k: allele number; Ho: observed heterozygosity; He: expected heterozygosity; AR: allele richness; HW: *p<0.05, **p<0.01, NS; LD: linkage disequilibrium, representing the number of linked loci. PIC: polymorphic information content.

Table S8. Genetic variation and polymorphism of 20 EST-SSR loci in *Hynobius arisanensis*.

Locus	N	k	Ho	He	PIC	LD	HW	AR
Locus13	30	4.000	0.300	0.456	0.409	0.000	*	5.456
Locus26	30	7.000	0.667	0.809	0.766	1.000	*	7.205
Locus30	30	4.000	0.267	0.714	0.647	2.000	**	6.485
Locus52	30	4.000	0.433	0.516	0.448	0.000	NS	2.995
Locus58	30	7.000	0.467	0.569	0.514	0.000	**	1.955
Locus60	30	7.000	0.172	0.624	0.582	2.000	**	1.869
Locus76	30	6.000	0.633	0.724	0.665	0.000	NS	6.593
Locus79	30	2.000	0.067	0.127	0.117	0.000	NS	2.000
Locus87	30	2.000	0.067	0.127	0.117	0.000	NS	8.421
Locus88	30	5.000	0.200	0.650	0.570	0.000	**	7.854
Locus89	30	3.000	0.267	0.501	0.387	1.000	**	2.985
Locus91	30	9.000	0.433	0.801	0.768	1.000	**	4.739
Locus94	30	3.000	0.333	0.501	0.420	0.000	**	2.985
Locus96	30	4.000	0.200	0.295	0.270	0.000	*	3.252
Locus104	30	5.000	0.233	0.424	0.394	0.000	**	2.588
Locus105	30	4.000	0.233	0.327	0.305	0.000	NS	4.493
Locus108	30	3.000	0.167	0.269	0.247	0.000	*	3.868
Locus114	30	4.000	0.233	0.629	0.567	0.000	**	1.999
Locus123	30	3.000	0.067	0.128	0.121	0.000	NS	2.955
Locus131	30	3.000	0.033	0.158	0.147	1.000	**	1.955
mean	30	4.450	0.274	0.467	0.423	0.400	-	4.13

N: sample number; k: allele number; Ho: observed heterozygosity; He: expected heterozygosity; AR: allele richness; HW: *p<0.05, **p<0.01, NS; LD: linkage disequilibrium, representing the number of linked loci. PIC: polymorphic information content.

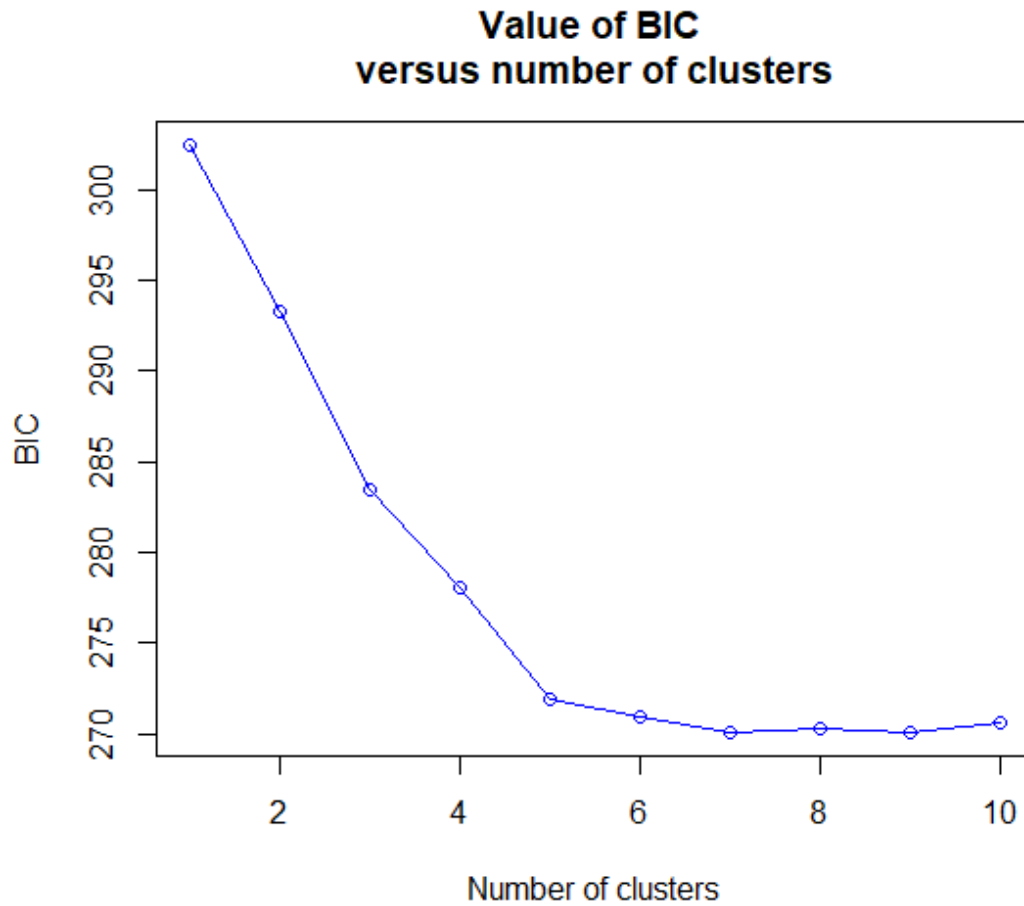


Figure S1. Bayesian Inference Criterion (BIC) values versus numbers of clusters (K), indicating that $K = 5$ is the most likely number of genetically distinct clusters.

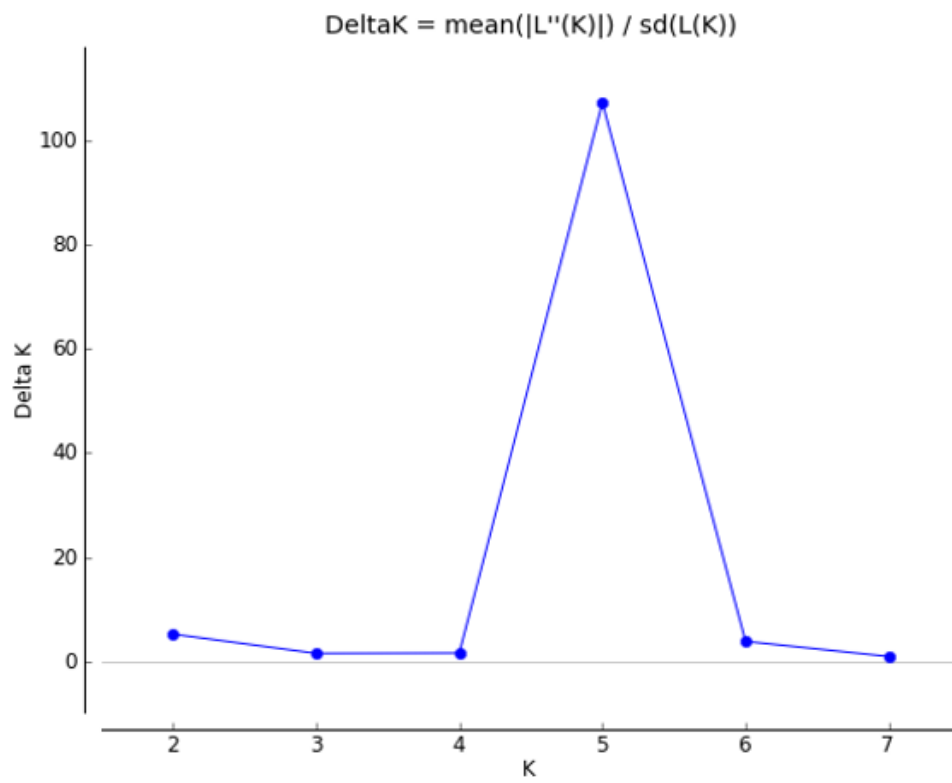


Figure S2. Estimating the optimal number of clusters (K) from an assumed range of 1–7, indicating that $K = 5$ is the most likely number of genetically distinct clusters.