

Supplementary Materials

Phylogeographical Analysis of the Freshwater Gudgeon *Huigobio chenhsienensis* (Cypriniformes: Gobionidae) in Southern China

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Table S1. Reviews of the genetic diversity of freshwater fishes endemic to southern China revealed by mitochondrial genes

Category	Order	Species	Basin	h	π	References
II	Cypriniformes	<i>Sinibrama macrops</i>	Qiantang River, Cao'e River	0.9610	0.0044	[72]
		<i>Schizothorax nukiangensis</i>	Nujiang River	0.9650	0.0024	[73]
		<i>Acrossocheilus longipinnis</i>	Pearl River	0.6859	0.0036	[74]
IV	Cypriniformes	<i>Aphyocypris normalis</i>	Pearl River, Jianjiang River, Moyang River, Beilun River, Nandu River, Wanquan River, Zhubijang River, Lingshui River, Changhua River	1.0000	0.0149	[14]
		<i>Onychostoma lepturum</i>	Nandu River, Wanquan River, Lingshui River, Changhua River	0.9420	0.0229	[17]
		<i>Tanichthys albonubes</i>	Luo River, Pearl River, Beilun River, Red River, Changhua River	0.9700	0.0516	[75]
		<i>Poropuntius huangchuchieni</i>	Mekong River, Red River, Nujiang River	0.9856	0.0287	[11]
		<i>Garra orientalis</i>	Min River, Jiulong River, Pearl River, Moyang River, Wanquan River, Nandu River, Changhua River	0.9810	0.0074	[18]
		<i>Opsariichthys hainanensis</i>	Tan River, Moyang River, Jianjiang River, Nanliujiang River, Red River, Longwei River, Longshou River, Lingshui River, Wanquan River, Nandu River, Changhua River	0.9460	0.0149	[76]
	Perciformes	<i>Coreoperca whiteheadi</i>	Yangtze River, Qiantang River, Moyang River, Pearl River	0.8048	0.0601	[15]
	Siluriformes	<i>Glyptothorax fokiensis</i>	Yangtze River, Min River, Jiulong River, Hanjiang River, Pearl River, Moyang River	0.9920	0.0406	[13]
		<i>Glyptothorax hainanensis</i>	Jianjiang River, Lianjiang River, Nanliujiang River, Wanquan River	1.0000	0.0188	[13]

Table S2. Reviews of the phylogeographical patterns of freshwater fishes endemic to southern China revealed by mitochondrial genes

Pattern	Order	Species	References
I	Cypriniformes	<i>Hemibagrus guttatus</i>	[65]
		<i>Aphyocypris normalis</i>	[14]
		<i>Tanichthys albonubes</i>	[75]
		<i>Poropuntius huangchuchieni</i>	[11]
	Perciformes	<i>Coreoperca whiteheadi</i>	[15]
	Siluriformes	<i>Glyptothorax fokiensis</i>	[13]
<i>Glyptothorax hainanensis</i>		[13]	
II	Cypriniformes	<i>Schizothorax nukiangensis</i>	[73]
		<i>Acrossocheilus longipinnis</i>	[74]
		<i>Onychostoma lepturum</i>	[17]
		<i>Opsariichthys hainanensis</i>	[76]
	Siluriformes	<i>Hemibagrus macropterus</i>	[16]
V	Cypriniformes	<i>Garra orientalis</i>	[18]

Table S3. Primers and annealing temperature used in PCR amplification of mitochondrial genomes

Fragment	Gene	Site	Name of primer	Sequence (5'-3')	Source	Annealing temperature
1	12S-16S	72-1479	T-Gob12SF	AAGGCATGGTCCYGACCTTA	[27]	55.4 °C
			CGob16sR	TTCGGTAGGTCTRTCACTTC	[27]	
2	Val-ND1	1066-2870	CGobValF	ACACCGAGAAGACATCCA	[77]	54.3 °C
			CGobLeuR1	GGAAGAGGAYTTGAACC	[27]	
3	16S-ND2	2572-4072	CGobND1F	GCAGCCGCTATTAAGGGTT	[27]	52.3 °C
			CYMPHND1R	TTTATTATGAGAATGTTGGC	This study	
4	Ile-COI	3870-5570	T-SD-IleF	GCCCAAGGACCACTTTGATAG	[27]	52.5 °C
			CGobCo1R2	CCAAATACRAGATARAGGGT	[77]	
5	Asn-Ser	5312-7109	LY-AsnF	AGCGAGCATCCATCTACTT	[27]	54.6 °C
			CARSerR	GGTYATGTGACTGGCTTGA	[27]	
6	COI-ATP6	6958-8866	TGobCOIF	TGAGAAGCCTTYGCCGCYAAACG	[27]	52.4 °C
			TGobATP6R	GCTTGGTGTGCCATTARACGTTTTCTTG	This study	
7	ATP6-Arg	8528-10066	ZXSATP6F	AATCAACCAACMGTAGCYCT	This study	53.3 °C
			CGobArgR	CTGAGYCGAAATCAGAGG	[27]	
8	Gly-ND4	9665-11020	CGobGlyF	CTTCCAATYATTTAGYCTTGG	[77]	53.5 °C
			NMIND4R	TGCAACAGGGGCYTCTAC	This study	
9	ND4L-Leu	10660-12010	TGobND4FS	TAGCCAGCCAAAAYCACAT	This study	54.5 °C
			CGobLeuR	TGGAYTTGCACCAAGAGT	[27]	

Table S3 (*continued*)

Fragment	Gene	Site	Name of primer	Sequence (5'-3')	Source	Annealing temperature
10	His-ND5	11822-13375	CGobSerF1-M	ACYCACCRAGGAAGGACA	This study	50.0 °C
			CYND5R	ATAATTGAYCCYGAGCA	This study	
11	ND5-CYTB	12949-14471	TGobND5F	GTAGCTTTCTCRACATC	This study	50.5 °C
			NMICYTBR	CGTAGGCTTGCCATTATT	This study	
12	ND6-Pro	14322-15680	CGluF	TCTTGCTCGGAYTTTAACCGAG	[26]	58.0 °C
			CThrR	CCTCCGATCTTCGGATTACAAG	[26]	
13	Thr-12S	15566-217	HemDloopF	AAAGCATCGGTCTTGTAATC	[27]	53.0 °C
			HemDloopR	CTTGGCTAGGCGTCTTGG	[27]	

Table S4. Sampling localities and source of mitochondrial genome for *Huigobio* and outgroup taxa

Species	Locality	Genbank accession number	References
<i>Huigobio</i>			
<i>Huigobio chenhsienensis</i>	Xinchang, Zhejiang Province, China	ON316823	This study
<i>Huigobio exilicauda</i>	Shixing, Guangdong Province, China	ON316824	This study
<i>Huigobio heterocheilus</i>	Yanling, Hunan Province, China	ON316825	This study
<i>Microphysogobio</i>			
<i>Microphysogobio jeon</i>	Buyeo-gun, South Korea	MN581867	[78]
<i>Microphysogobio springeri</i> (=Abbottina <i>springeri</i> = <i>Biwia springeri</i>)		AP011360	[37]
<i>Microphysogobio brevirostris</i>		KF319122	[79]
<i>Microphysogobio koreensis</i>	South Korea	FJ515920	[80]
<i>Microphysogobio longidorsalis</i>	South Korea	AP011394	[37]
<i>Microphysogobio rapidus</i>	South Korea	MH713708	[81]
<i>Microphysogobio alticorpus</i>		KC762939	[82]
<i>Biwia</i>			
<i>Biwia zezera</i>	Gifu, Japan	AB250107	[83]
<i>Platysmacheilus</i>			
<i>Platysmacheilus exiguus</i>	Yangsuo, Guangxi Zhuang Autonomous Region, China	ON316826	This study
<i>Gobiobotia</i>			
<i>Gobiobotia macrocephala</i>	South Korea	FJ515918	[84]
<i>Xenophysogobio</i>			
<i>Xenophysogobio nudicorpa</i>	Jiangjin, Chongqing Municipality, China	KU314698	[85]

Table S4 (continued)

Species	Locality	Genbank accession number	References
<i>Hemibarbus</i>			
<i>Hemibarbus nummifer</i> (= <i>Belligobio nummifer</i>)	Wuyishan, Fujian Province, China	KJ413052	[86]
<i>Hemibarbus longirostris</i>	Ganwon-do, South Korea	DQ347952	[87]
<i>Squalidus</i>			
<i>Squalidus longifilis</i>	Fengcheng, Liaoning Province, China	MT767747	[27]
<i>Abbottina</i>			
<i>Abbottina binhi</i>	Pingguo, Guangxi Zhuang Autonomous Region, China	MK852688	[88]
<i>Pseudogobio</i>			
<i>Pseudogobio anderssoni</i>	Anyi, Jiangxi Province, China	MN883563	[89]
<i>Pseudogobio guilinensis</i>	Pingle, Guangxi Zhuang Autonomous Region, China	MN883564	[89]
<i>Saurogobio</i>			
<i>Saurogobio dabryi</i>	Xiushui, Jiangxi Province, China	KU314696	[85]
<i>Saurogobio gymnocheilus</i>	Hengdong, Hunan Province, China	MK860911	[90]
<i>Gobio</i>			
<i>Gobio acutipinnatus</i>	Bu'erjin, Xinjiang Uygur Autonomous Region, China	MT632635	[91]
<i>Gobio gobio</i>	Plana	AB239596	[92]
<i>Mesogobio</i>			
<i>Mesogobio lachneri</i>	Linjiang, Jilin Province, China	OL678457	unpublished (from Genbank)
<i>Acanthogobio</i>			
<i>Acanthogobio guentheri</i>		MF787799	unpublished (from Genbank)
<i>Romanogobio</i>			
<i>Romanogobio ciscaucasicus</i>		AP011259	unpublished (from Genbank)

Table S4 (continued)

Species	Locality	Genbank accession number	References
<i>Gnathopogon</i>			
<i>Gnathopogon elongatus</i>	Japan	AB218687	[92]
<i>Gnathopogon imberbis</i> (= <i>Gnathopogon polytaenia</i>)		KM086722	[93]
<i>Coreoleuciscus</i>			
<i>Coreoleuciscus splendidus</i>	South Korea	EU848546	[94]
<i>Coreoleuciscus aeruginos</i> *	South Korea	DQ347951	[94]
<i>Pseudorasbora</i>			
<i>Pseudorasbora elongata</i>	Wuyuan, Jiangxi Province, China	KF245485	[95]
<i>Pungtungia</i>			
<i>Pungtungia herzi</i>	South Korea	KF006339	[96]
<i>Pseudopungtungia</i>			
<i>Pseudopungtungia nigra</i>	South Korea	EU332752	[97]
<i>Sarcocheilichthys</i>			
<i>Sarcocheilichthys parvus</i>	Yongjia, Zhejiang Province, China	JX456224	[98]
<i>Sarcocheilichthys nigripinnis</i>	Kaihua, Zhejiang Province, China	JX401522	[99]
<i>Paracanthobrama</i>			
<i>Paracanthobrama guichenoti</i>		KJ645748	[100]
<i>Coreius</i>			
<i>Coreius guichenoti</i>	Banan, Chongqing Municipality, China	JF906108	[101]
<i>Coreius heterokon</i>	Banan, Chongqing Municipality, China	JF906110	[101]
<i>Ladislavia</i>			
<i>Ladislavia taczanowskii</i>	Kuandian, Liaoning Province, China	MT897995	[102]

Table S4 (*continued*)

Species	Locality	Genbank accession number	References
<i>Acheilognathus</i>			
<i>Acheilognathus macropterus</i>	Wuhan, Hubei Province, China	KJ499466	[103]
<i>Rhodeus</i>			
<i>Rhodeus sericeus</i>	Xiaoxing'anling, Heilongjiang Province, China	KM052222	[104]
<i>Tanakia</i>			
<i>Tanakia limbata</i>	Okayama, Japan	KM386633	[105]

*The scientific name in Genbank is *Coreoleuciscus splendidus* that it is revised as *Coreoleuciscus aeruginos* by Song and Bang [106].

Table S5. Comparisons among six models of biogeography analyses in ancestral area reconstruction of *H. chenhsienensis*

Model	LnL	Parameter			Weight	
		<i>d</i>	<i>e</i>	<i>j</i>	AIC_wt	AICc_wt
DEC	-30.11	1.80E-02	1.00E-12	0.00	4.60E-03	5.07E-03
DEC+J	-25.77	1.00E-12	1.00E-12	5.48E-03	1.29E-01	1.29E-01
DIVALIKE	-32.23	3.08E-02	8.47E-03	0.00	5.51E-04	6.08E-04
DIVALIKE+J	-23.87	1.00E-12	1.00E-12	5.40E-03	8.65E-01	8.65E-01
BAYAREALIKE	-50.07	4.77E-02	4.01E-01	0.00	9.80E-12	1.08E-11
BAYAREALIKE+J	-31.56	1.00E-07	8.35E-02	8.72E-03	3.97E-04	3.97E-04

The optimal model is highlighted in bold based on LnL and weight values.