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Short Communication

Mitochondrial diversity and phylogeography of the Chinese perch, *Siniperca chuatsi* (Perciformes: Sinipercidae)

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1. Introduction

Siniperchids are a small group of freshwater perciform fish endemic in East Asia that have attracted much attention from taxonomists. Although challenged by a recent molecular study using cytochrome *b* gene sequences (Chen et al., 2007), most morphological (Liu and Chen, 1994) and molecular evidences (Zhang et al., 2006; Zhao et al., 2006a) support the monophyly of siniperchids, including two sister genera, *Coreoperca* with three species and *Siniperca* with six species (Li, 1991).

Siniperca chuatsi, commonly known as the Chinese perch or the mandarin fish, is the most famous siniperchid species because it is not only an important economical species but also a symbol in the rich Chinese fish culture whose name appeared in many ancient Chinese poems and books. *S. chuatsi* is distributed in every major river north of the Zhujiang (Pearl) River in eastern China (Zheng, 1989). Because of overfishing, drought and especially water pollution, *S. chuatsi* is declining in many areas recently (Zhang and Zhao, 1999). Since *S. chuatsi* is a strict freshwater species, the vicariance of the species with different river drainages could lead to long-term genetic isolation and divergence. Underestimation of the within-species diversity has been reported in other freshwater fishes distributed in the same drainages of eastern China (e.g., *Perdices* et al., 2005). It is possible that the genetic diversity of *S. chuatsi* also is underestimated, so the genetic structure of *S. chuatsi* among different river drainages is in urgent needs for evaluation given the increasing conservational concerns to this species.

The distribution of *S. chuatsi* spans over two major zoogeography regions (Palearctic Region and Oriental Region), so another profit from studying genetic divergence of *S. chuatsi* is that it can

be used to explicitly test biogeography hypotheses about the division of freshwater ichthyofauna in eastern China. Based on the similarity of species composition between different river drainages, Mori (1936) took the Nanling Mountain Range as the demarcation line between Palearctic Region and Oriental Region (Fig. 1). This view was shared by many subsequent studies (e.g., Li, 1981; Xu et al., 2002). However, Chen and Cao (1986) questioned the validity of using similarity of species composition as the criterion for dividing regions and proposed the Qinling-Dabie Mountain Range as the demarcation line between Holarctic Region and Oriental Region in East Asia (Fig. 1). They argued that a simple comparison of overall species similarity was inadequate or even misleading for delimiting faunal division. Although the issue about where to draw the demarcation line between Holarctic Region and Oriental Region in eastern China is still under debate (Xu et al., 2002; Zhang and Chen, 1997), no molecular data of fish species have been used to test the competing hypotheses.

The objectives of this study are: (1) to assess the phylogeny and genetic divergence of *S. chuatsi* among the major drainages and explicitly test the hypotheses about the demarcation line separating Palearctic Region and Oriental Region in eastern China; (2) to estimate genetic diversity and other demographic parameters of each population to provide genetic background data for conservational practice.

2. Materials and methods

2.1. Sample collection

A total of 79 samples of *S. chuatsi* were collected from eight localities of five rivers, the Heilongjiang River (HL), the Huaihe River (HH), the Changjiang River (CJ), the Qiantangjiang River (QT) and the Minjiang River (MJ). In the Changjiang River, four localities were sampled, Dongting Lake (DT), Poyang Lake (PY), the Qiupu

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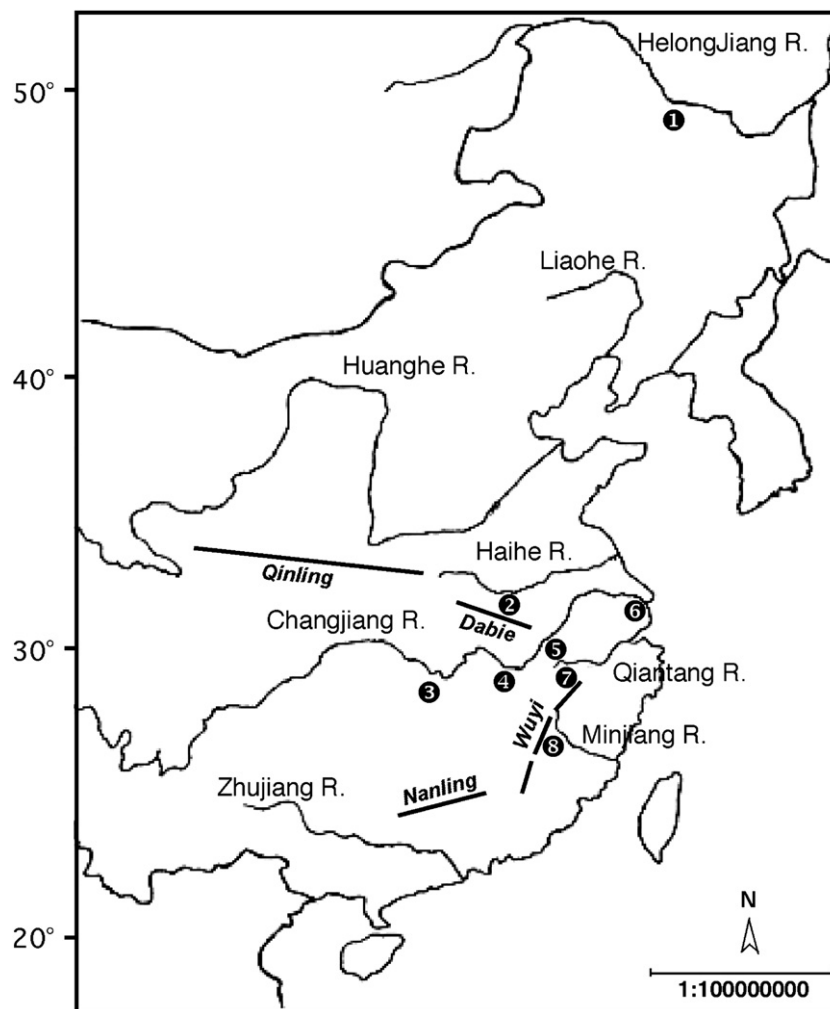


Fig. 1. Sampling locations. The black bars show the mountain ranges being assumed as the demarcation lines (Qinling–Dabie or Nanling–Wuyi) separating Palearctic Region and Oriental Region.

River (QP) and Taihu Lake (TH) (Fig. 1; Table 1). Efforts to collect samples from the Huanghe River and the Liaohe River turned out to be futile because of the rarity of *S. chuatsi* in these rivers in recent years.

2.2. PCR amplification and sequencing

Caudal fins (20–30 mg) were minced and digested with Proteinase K. Total DNA was isolated using standard phenol–chloroform extraction. The PCR primers and condition followed Zhao et al. (2006b). The amplified DNA fragments were sequenced on an automatic DNA Sequencer (ABI PRISM 377, Perkin-Elmer) following the manufacturer's protocols.

2.3. Sequence alignment and phylogenetic analyses

Identical sequences were found and collapsed using Collapse v1.2 (Posada, 2006). Control region sequences of *S. kneri* (EU659699), *S. obscura* (EU659702), *S. scherzeri* (EU659700), *S. undulata* (EU659704), *S. roulei* (EU659706) and *Coreoperca whiteheadi* (EU659708) were used as the outgroups (Zhao et al., 2006b). Sequences of *C. kawamebari* (AP005990), *Perca fluviatilis* (Y14724), *Oryzias latipes* (NC_004387) and *Gasterosteus aculeatus* (AB054361) were retrieved from GenBank for dating the nodes of the resulting phylogenetic tree. Multiple alignments of the

Table 1
Populations, collection sites and numbers of individuals sampled

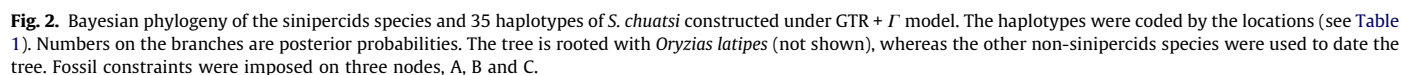
Site No.	Population code	Collection site	Longitude/latitude	No. of Samples
1	HL	Xunke, Heilongjiang	128°42'E, 49°57'N	9
2	HH	Huainan, Anhui	117°02'E, 32°65'N	9
3	DT	Yuanjiang, Hunan	112°36'E, 28°83'N	10
4	PY	Yongxiu, Jiangxi	115°82'E, 29°05'N	11
5	QP	Chizhou, Anhui	117°48'E, 30°66'N	11
6	TH	Suzhou, Jiangsu	120°54'E, 31°29'N	10
7	QT	Chunan, Zhejiang	119°05'E, 29°61'N	10
8	MJ	Shunchang, Fujian	117°80'E, 26°80'N	9

* Site number see Fig. 1.

sequences were performed using the Clustal W (Thompson et al., 1994).

ModelTest v3.7 (Posada and Crandall, 1998) was used to compare the different candidate models and the best model was selected based on the Akaike Information Criterion (AIC). Bayesian analysis with the selected model was carried out using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003).

To date the nodes splitting *Siniperca* species and the major nodes within *S. chuatsi*, MULTIDIVTIME (Thorne and Kishino, 2002) was used. Three calibration points were used. The splitting of stickleback and medaka (node A in Fig. 2) was set as older than



To test the biogeography hypothesis about the boundary of ichthyofauna division, the methods to identify genetic barriers based on the data, spatial analysis of molecular variance (SAMOVA; [Dupanloup et al., 2002](#)) was used. This approach is based on a simulated annealing procedure that aims at maximizing the proportion of total genetic variance due to differences between groups of populations ([Dupanloup et al., 2002](#)). We ran SAMOVA with

The nucleotide diversity and haplotype diversity were estimated for each population using Arlequin v 3.1 (Excoffier et al., 2005). Tajima's D test and Fu's F_s test implemented in Arlequin v3.1 were used to check the neutrality of the gene sequence. Mismatch distribution test implemented in Arlequin v3.1 was carried out for each population against the null hypothesis of population expansion. The genetic distances among populations of different rivers as well as among the populations within the Changjiang River were calculated using Arlequin v3.1. The corrected net average pairwise difference (D_A), which equals the between population distance minus the within population distance was estimated.

To assess the divergence of populations within the Changjiang River, analysis of molecular variance (AMOVA) was performed using Arlequin v3.1 to evaluate the genetic variance among populations. A Mantel test on the correlation between genetic distance (pairwise F_{ST}) and geographic distance among populations also was performed to test the null hypothesis of isolation by distance in the Changjiang River.

3. Results

3.1. Sequence characterization, phylogenetic relationships and molecular dating

The length of control region sequences obtained was 785 bp. There were 35 unique haplotypes from the 79 individuals sequenced. The unique haplotypes were deposited in GenBank (EU659709–EU659743).

The GTR + Γ model was selected based on the AIC values using ModelTest. The sister-group relationship between *Siniperca* and *Coreoperca* was supported with a posterior probability (pp) of 0.96 in the Bayesian analysis. The monophyly of *Siniperca* also was supported with a pp value of 0.97 (Fig. 2). Within *Siniperca*, *S. chuatsi* was placed as the basal species to a clade that included all of the other *Siniperca* species except for *S. kneri*, which was found embedded within the clade of *S. chuatsi* (Fig. 2). *Siniperca scherzeri* is basal to *S. undulata*, which is basal to the *S. obscura* and *S. roulei* (Fig. 2). The individual haplotypes of *S. chuatsi* from different rivers have not been sorted out into reciprocal monophyletic groups. However, all the haplotypes from the north group (HL and HH, north of the Qinling-Dabie Mountain Range) formed a monophyletic clade, which made the haplotypes from the south rivers (CJ, QT and MJ, south of the Qinling-Dabie Mountain Range, Fig. 1) basal and paraphyletic to the north group. No structure was found within the haplotypes of south populations (Fig. 2).

Calibrated by three constraining nodes (A, B and C in Fig. 2) on the resulting phylogeny, the divergence time between *Siniperca* and *Coreoperca* (node d in Fig. 2) was estimated as 4.36 (7.80–2.81) MYA, while the age of the most recent common ancestor of all *Siniperca* (node C in Fig. 2) was found to be 3.8 (6.93–2.54) MYA (Fig. 2). Since the north populations of *S. chuatsi* formed a monophyletic group, the splitting of this group from the basal south populations should occur between the most recent common node of the north and the south haplotypes (node h in Fig. 2) and the coalescent node of all north haplotypes (node i in Fig. 2). The age of node “h” is 1.57 (3.51–0.55) MYA, whereas the age of node “i” is 1.27 (2.99–0.39) MYA. Therefore, the north populations should diverge from the south populations during the period of 3.51 to 0.39 MYA.

3.2. Divergence between drainages, SAMOVA and gene flow

The net average pairwise population distances (D_A) between north rivers and the south rivers are 3.77–8.22 with a mean of 5.97, while the genetic distances between rivers within the north and the south group are 0.10–3.40 (mean = 1.34).

The SAMOVAs obtained the highest F_{CT} when all populations were divided into the north (HL and HH) and the south (DT, PY, QP, TH, QT and MJ) groups along the Qinling-Dabie Mountain Range, suggesting that the biggest genetic gap in *S. chuatsi* exists along the division of Qinling-Dabie Mountain Range (Fig. 1).

Gene flows between adjacent rivers estimated using IMA are listed in Table 2. Both the marginal peak values and the means of marginal posterior probability have the lowest values of the gene flow between the Huaihe River (HH) and the Changjiang River (CJ, including DT, PY, QP and TH). The means of gene flow between

Table 2

Gene flow between adjacent rivers estimated using the isolation by migration model, IMA

Gene flow direction	Marginal peak locations	Mean ^a	95% CI
HL→HH	0.2402	1.272	0.063–4.473
HH→HL	0.0001	0.516	0.009–1.807
HH→CJ	0.0001	0.044	0.001–0.182
CJ→HH	0.0001	0.060	0.001–0.226
CJ→QT	0.5144	1.945	0.093–4.718
QT→CJ	0.0001	0.541	0.015–1.757
QT→MJ	0.0001	1.191	0.028–4.293
MJ→QT	0.2616	1.109	0.043–3.988

^a Mean of marginal posterior probability.

the Huaihe River and the Changjiang River are at least one fold less than the means of gene flow between the other rivers (Table 2).

3.3. Genetic diversity of populations and differentiation within the Changjiang River

Gene diversity ranges from 0.417 in MJ to 0.917 in HH among the eight populations. The lowest nucleotide diversity is also in MJ (0.001), while the populations with highest nucleotide diversity are PY (0.009) and HH (0.008). Both Tajima's D test and Fu's F_S failed to reject the neutrality of sequences in most populations, except for HL and MJ ($P < 0.05$), suggesting possible recent population expansion in these rivers (results not listed). The null hypothesis of recent population expansion could not be rejected in most population by the mismatch distribution test except for DT and QT ($P < 0.05$).

Surprisingly, the distances between populations with the Changjiang River are 0.81–3.15 (mean = 1.37), in the same range as the distances between rivers within the north and south region. The results of AMOVA shows that the genetic variation among rivers is 32.25%, while the variation among populations within rivers is 16.37% and the within population variation is 51.38%. The AMOVA for the populations within the Changjiang River only also revealed 21.53% variation attributed to among populations.

The correlation between the genetic distance and the geographic distance among the populations in the Changjiang River was not supported by the Mantel test ($P = 0.349$), suggesting that the genetic divergence between populations in the Changjiang River cannot simply be explained as the isolation by distance.

4. Discussion

4.1. Phylogeny of sinipercids and population divergence of *S. chuatsi* among rivers

The monophyly of Sinipercidae was supported in our results ($pp = 0.96$). The monophyly of *Coreoperca* and *Siniperca* also were highly supported by our data with the pp values of 1.00 and 0.97, respectively (Fig. 1). Chen et al. (2007) discovered the non-monophyly of Sinipercidae with mitochondrial Cytb sequence data; however, other studies using Cytb gene sequences did not support their conclusion (Zhang et al., 2006; Zhao et al., 2006a). The inconsistency among these studies justifies further investigation on this issue and probably a multi-locus nuclear dataset would provide a resolution. The most recent common ancestor of all *Siniperca* species was dated as 3.8 MYA with *S. chuatsi* as the basal branch (Fig. 2). The basal position of *S. chuatsi* was reported by another study using Cytb gene sequences (Zhao et al., 2006a), but not by a morphological study (Liu and Chen, 1994). However, all studies agreed on the sister-species relationship between *S. chuatsi* and *S. kneri* (Chen et al., 2007; Liu and Chen, 1994; Zhang et al.,

2006; Zhao et al., 2006a). In this study, *S. kneri* was found embedded in the clade of *S. chuatsi* (Fig. 2), suggesting their close relationship and probably a recent speciation event between these two species.

In the classic view of the ichthyofauna division in eastern China, Palearctic Region and Oriental Region split along the Nanling-Wuyi Mountain Range (Li, 1981; Mori, 1936), and it is still followed by some recent studies (e.g., Xu et al., 2002). The alternative hypothesis proposed the Qinling-Dabie Mountain as the demarcation line between Palearctic Region and Oriental Region (Chen and Cao, 1986; Zhang and Chen, 1997). In this study, the genetic divergence among the populations of *S. chuatsi* in different rivers supports Qinling-Dabie as the division of ichthyofauna in eastern China. Firstly, the results of SAMOVA reveal that the biggest genetic gap among populations of *S. chuatsi* lies along with the Qinling-Dabie Mountain Range. Secondly, the estimated gene flow between adjacent rivers also suggests that the exchange across the Qinling-Dabie Mountain Range is the least (Table 2). Thirdly, direct comparison the genetic distances between rivers showed that the divergences between the south and the north rivers are much higher than the divergences between rivers within the south or north region. Finally, the estimated date when the north population diverged from the south population was from 3.51 to 0.39 MYA (Fig. 2), which is consistent with that of the Qinling-Dabie orogen formed during Mesozoic–Cenozoic (Sun et al., 2004). All of the evidences point out that the Qinling-Dabie Mountain Range probably is the most important barrier for ichthyofauna in eastern China, at least the main force sculpting the geographic structure of *S. chuatsi*. More species living in the same region should be investigated to see if the same pattern holds.

4.2. Genetic diversity within *S. chuatsi*, population differentiation in the Changjiang River

Historically, *S. chuatsi* was abundant in large rivers and lakes in China. Due to climate change, water pollution and overfishing, the total numbers of *S. chuatsi* decreased dramatically in the second half of the last century. They almost disappeared completely in northern China because of long time draught (Zhang and Zhao, 1999). The genetic diversity and population structure of *S. chuatsi* are vital information for conservational practice for this species.

Our study shows that the genetic diversity of *S. chuatsi* is moderate compared to other *Siniperca* species (Wang et al., 2006; Zhao et al., 2007). However, the genetic divergence between populations is high, with 32.25% variance attributed to among rivers and 16.37% variance attributed to among populations within rivers. The high divergence between rivers, especially no haplotypes shared between the south and north regions suggest that the diversity within *S. chuatsi* should not be underestimated. When conservational policies are made, the diverged populations with high diversity, such as HH, PY should all be given high priority.

Finally, our results showed that the population differentiation within the Changjiang River is high. The genetic distances between the four populations in the Changjiang River are as large as the distances between populations from different rivers in the north or south regions. The genetic variance among populations is 21.52% when only the four populations in the Changjiang River were analyzed, which is much higher than 4.4%, the number from a similar study on grass carp in the Changjiang River (Liao et al., 2005).

The pattern of population divergence suggests high fragmentation of *S. chuatsi* in the Changjiang River and the limited effects of dispersal. Another interesting result is that the isolation by distance pattern in the Changjiang River was rejected by the Mantel

test. Other hypotheses, such as river capture or translocation for aquaculture purpose could explain the pattern but need further examination.

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