INTRODUCTION

The Yangtze River is the longest river in China and the third longest in the world, and it occupies various types of ecosystems. According to the river characteristics, the river section above Yichang City, Hubei Province, is considered the upper reaches, and the section below Yichang City the middle and lower reaches. There are approximately 361 fish species inhabiting the river basin, 177 of which are endemic (Fu, Wu, Chen, Wu, & Lei, 2003). Distribution patterns across the river are different for different species, and many fishes are only endemic in special sections or tributaries. Consequently, the genetic structures among populations are important for interpreting adaptation to heterogeneous environments. Additionally, the Pleistocene glaciations have had profound effects on the phylogeography and historical demography of species (Dynesius & Jansson, 2000; Hewitt, 2000). Freshwater fishes were restricted in rivers or lakes and experienced a fluctuant landscape of intermittently connected and isolated watersheds and glacial refugia, which provides many opportunities to study phylogeography and speciation (Ruskey & Taylor, 2016; Ruzzante, 2016).
Walde, Macchi, Alonso, & Barriga, 2011; Taylor, 1999; Zhou, Song, Wang, Jie, & Gao, 2016).

The subfamily Gobiobotinae (Cyprinidae, Cypriniformes) is a group of small freshwater gudgeon distributed in the rivers of East Asia in Korea and China (Chen, 1998). Only two genera, Gobiobotia and Xenophysogobio, with 17 species belong to this subfamily, and all the fishes are benthic. He (1991) examined the skeletons and classified Xenophysogobio as a primitive species and Gobiobotia filifer as a specialized species. Wang, He, and Chen (2002) used mitochondrial DNA (mtDNA) data to construct the phylogenetic relationship that supported this idea. They also inferred that Gobiobotinae originated in the upper reaches of the Yangtze River. At present, four fishes, including X. boulegeri, X. nudicorpa, G. abbreviata, and G. filifer, live in the upper reaches of the Yangtze River. However, except for these four fishes, sympatric habitation among Gobiobotinae fishes is rare, indicating that they could have quickly adapted and specialized to new ecological environments.

Gobiobotia filifer is endemic to the Yangtze River basin (Chen, 1998), and the limits of its native range extend from the Yibin City to downstream, including tributary rivers such as Min River, Chishui River, Hanjiang River, and Xiangjiang River (Figure 1). This fish produces drifting eggs in spawning season (Liu et al., 2018; Tian et al., 2017) and feeds on benthic organisms. The Gezhouba Dam was completed in 1988 in Yichang City, and then, the Three Gorges Dam with 175 m of water deep was completed in 2006, which form physical and ecological barriers to genetic exchange among populations. Recent surveys found that the abundance of G. filifer in upper Yangtze River was relatively high comparing with that before the construction of the Three Gorges Dam (Fan, Ba, & Duan, 2012; Xiong, Liu, Duan, Liu, & Chen, 2015; Yang, Xin, Ma, Kong, & Liu, 2010). However, it was less common in section and tributary rivers below the dams now (Fan et al., 2012).

Own to the specialized position in phylogeny and relatively broad distribution, G. filifer is a good mode for studying the local adaptation and the effect of dam on fish. For the last decades, mitochondrial DNA has been proven to be an important tool in population history, biogeography, genetic structure, and species delimitation (Hebert, Penton, Burns, Janzen, & Hallwachs, 2004; Moore, 1995). In this study, we used cytochrome b (Cyt b) to examine population genetic diversity of G. filifer. We aimed to (a) reveal whether genetic divergence occurred within the population and (b) determine whether divergence occurred before or after population radiation.

### 2 | MATERIALS AND METHODS

#### 2.1 | Sampling and DNA extraction

A total of 292 individuals of G. filifer were collected from eight locations from 2014 to 2016; four locations were in the upper reaches of the Yangtze River and four in the middle reaches (Table 1; Figure 1). A small fin sample was clipped and preserved in 95% ethanol for each specimen.

Genomic DNA was extracted using an easy-DNA Kit (Omega) following the manufacturer's instructions.

#### 2.2 | PCR and sequencing

The cytochrome b (Cyt b) gene was amplified using polymerase chain reaction (PCR) with primers L14742 and H15915 (Xiao, Zhang, & Liu, 2001). Each 50 μl PCR contained 1–10 ng of template DNA, 5 μl of 10 × PCR buffer, 1 μl of dNTP mix (10 mM), 10 pmol of each primer, and 2 U of rTaq polymerase (TaKaRa). PCRs were conducted in a
thermal cycler (T100; Bio-Rad) with the following program: one cycle of denaturation at 95°C for 4 min; 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 90 s; and one final cycle at 72°C for 10 min. Successful PCR products were separated by electrophoresis on a 1.0% agarose gel and purified using the Agarose Gel Purification Kit (Qiagen).

Several samples with distinct phylogenetic clades were also used to test the existence of cryptic species using COI gene. The primers and PCR information were as Ward, Zemlak, Innes, Last, and Hebert (2005).

Purified products then were sequenced with an ABI PRISM 3730 sequencer. Sequencing primers were the same as those used for PCR amplification. All unique sequences have been deposited in GenBank.

2.3 Data analyses

Sequences were assembled by Lasergene v7.1 (http://www.dnastar.com/) and aligned with the Clustal X 1.81 program (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). Population genetic diversity was measured for all samples, sampling groups, and divergent groups by determining haplotype diversity (h) and nucleotide diversity (π) in the DnaSP 6.0 software (Rozas et al., 2017).

A median-joining haplotype network was constructed in Network v4.6 (http://www.fluxus-engineering.com/). Phylogenetic analysis of haplotypes was conducted using the neighbor-joining (NJ) method and Bayesian inference (BI). The NJ analysis with the Kimura 2-parameter distance method was carried out in MEGA 7.0 (Kumar, Stecher, & Tamura, 2016). The tree nodes and branch lengths were statistically tested using the bootstrap method with 1,000 replicates and an interior branch test, respectively. BI analysis was carried out using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Best-fit models for the Bayesian analysis were inferred by hierarchical likelihood ratio tests on the IQ-TREE Web server (http://iqtree.cibiv.univie.ac.at/).

Pairwise \( F_{ST} \) values and analysis of molecular variance (AMOVA) were used to assess the population configuration in Arlequin v3.5 (Excoffier & Lischer, 2010). The pairwise \( F_{ST} \) values between sites were calculated and assessed for significance by comparison with 10,000 permutations of data.

Migration patterns were estimated using the coalescent-based program MIGRATE-n v3.6.11, which estimates migration rates between two groups of populations—groups with four populations in upper reaches (UPG) and four populations in middle reaches (MPG). Estimation of parameters in MIGRATE was done using Bayesian approach (Beerli, 2006).

Historical demographic expansions were investigated with neutral tests, such as Tajima’s D (Tajima, 1989) and Fu’s Fs (Fu, 1997), and pairwise nucleotide mismatch distributions; all of these tests were implemented in DnaSP 6.0 (Rozas et al., 2017). Departures from neutrality of Fu’s Fs and Tajima’s D test indicate recent population expansions under assumptions of neutrality (Ramos-Onsins & Rozas, 2002). The demographic history of *G. filifer* was explored using mismatch analysis of Cyt b mitochondrial sequences. This method is based on the premise that

| Sampling location          | Abbreviation | Geographical coordinates | Sample size | Haplotype number | Haplotype diversity, \( H_d \) | Nucleotide diversity, \( \pi \) | Tajima’s D  | Fu’s Fs  |
|----------------------------|--------------|--------------------------|-------------|------------------|-------------------------------|----------------|------------|----------|
| Yibin City, Yangtze River  | YYB          | 28°76′N 104°63′E         | 67          | 18               | 0.877                         | 0.0076          | 0.9775     | 0.171    |
| Hejiang County, Yangtze River | YHJ        | 28°81′N 105°83′E         | 14          | 3                | 0.692                         | 0.0052          | 0.7419     | 6.439    |
| Jiangjin City, Yangtze River | YJJ         | 29°27′N 106°28′E         | 70          | 17               | 0.815                         | 0.0064          | 0.7364     | −0.109   |
| Chishui City, Chishui River | CCS         | 28°58′N 105°69′E         | 54          | 15               | 0.827                         | 0.0068          | 0.5177     | 0.474    |
| Jingzhou City, Yangtze River | YJZ         | 30°30′N 112°24′E         | 24          | 16               | 0.946                         | 0.0080          | −0.1841    | −3.530*  |
| Jianli County, Yangtze River | YJL         | 29°53′N 112°93′E         | 31          | 14               | 0.886                         | 0.0068          | −0.0845    | −0.928   |
| Honghu City, Yangtze River | YHH          | 30°07′N 114°01′E         | 18          | 10               | 0.935                         | 0.0083          | 0.8021     | 0.384    |
| Miluo City, Xiangjiang River | XXJ         | 28°85′N 112°89′E         | 14          | 13               | 0.989                         | 0.0088          | 0.2064     | −4.737** |
| HapGroup 1                  |              |                          | 203         | 34               | 0.803                         | 0.0020          | −2.1016*   | −27.182**|
| HapGroup 2                  |              |                          | 89          | 32               | 0.891                         | 0.0025          | −1.4959    | −27.454**|
| Total                       |              |                          | 292         | 66               | 0.893                         | 0.0072          | −0.736     | −28.076**|

*Significant level at \( p < .05 \).
**Extremely significant at \( p < .01 \).
3 | RESULTS

A Cyt b dataset of 1,009 bp in size was obtained, and a total of 57 polymorphic sites were detected including 14 singleton variable and 43 parsimony informative sites. Among 292 sequences, 66 unique haplotypes were determined. The mean haplotype diversity and nucleotide diversity were 0.893 (range from 0.692 to 0.989) and 0.0072 (range from 0.0052 to 0.0088; Table 1), respectively. Genetic distance among haplotypes ranges from 0.001 to 0.019 (Table S1).

Both of the phylogenetic topologies of 66 G. filifer mtDNA haplotypes generated with NJ and Bayesian inference were similar, which are presented with two distinct haplotype groups, HapGroup 1 and HapGroup 2 with high confidence values (Figure S1). The sample distributions in the groups were not consistent with the geographical locations, and the two populations could each be found at all locations (Table S2).

Haplotype median-joining network supported the phylogenetic tree result that all of the mtDNA haplotypes fell into two haplotype groups (Figure 2). All haplotypes from each group were separated by at least 11 mutation steps, whereas neighbor haplotypes differed by a maximum of four (HapGroup 1) and two (HapGroup 2) mutations within groups.

HapGroup 1 had 34 haplotypes and contained 203 individuals (69.5% of samples), whereas HapGroup 2 had 32 haplotypes and 89 individuals (30.5% of samples; Table S3). The proportion of sample size of HapGroup 1 to HapGroup 2 was 2.28, and there was no significant difference among the proportions in eight sampling locations with $p = .63$ for the t test (two-tailed) and $p = .93$ for the chi-square test (Table S3). Haplotypes 2 (63 individuals, 31.0%) and 1 (61, 30.0%) were the most common haplotypes in HapGroup 1, and haplotype 41 was the most common in HapGroup 2 (25 individuals, 28.1%). The former two haplotypes were shared by seven locations, and the last one was shared by six locations. No single haplotype was found in all locations. The haplotype diversity and nucleotide diversity of HapGroup 1 and HapGroup 2 were 0.803 and 0.0020 and 0.891 and 0.0025, respectively.

The global AMOVA showed that no significant differentiation occurred among geographical populations with $F_{ST} = 4.55%$ (Table 2). Pairwise $F_{ST}$ values further revealed that genetic differences between YHJ and other populations were moderately large and significant. The divergence between XXJ and the four populations in the upper reaches was also significant (Table 3). The migration rates described genetic migration patterns dominated by asymmetric gene flow which UPG supplies much more migrants to MPG than otherwise ($M_{UPG-MPG} = 869.7$; $M_{MPG-UPG} = 86.8$; Table 4).

The local and global neutral test showed that only HapGroup 1 had a significantly negative value for Tajima’s $D$, and three populations, including XXJ, HapGroup 1, and HapGroup 2, and the total sample had highly significant negative values for Fu’s $F_{S}$ (Table 1). We further tested the two HapGroups with mismatch distribution under the sudden expansion model and found similar $r$ values for the HapGroup populations. Based on $\mu = 0.76%$ for cyprinid fish Cyt b gene (Zardoya & Doadrio, 1999), the expansion times were estimated at approximately 14.3 (6.1–38.0, CI = 95%) and 16.5 (8.3–20.6, CI = 95%) 1,000 years ago for the HapGroup 1 and HapGroup 2 populations, respectively (Table 5).

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FIGURE 2 A median-joining network of 66 Cyt b haplotypes of Gobiofilipta filifer in this study. The circle size is proportional to the haplotype frequency. The black dots indicate mutation steps.
Eighty-five COI sequences with 570 bp in size were also obtained and identified to 11 unique haplotypes. Genetic distance among haplotypes ranges from 0.002 to 0.022 (Table S4). The NJ tree constructed from the haplotypes was shown similar topology as that of Cytb (Figure S2).

### DISCUSSION

#### 4.1 Genetic diversity and structure

MitDNA Cyt b gene sequences have been used to study population genetics of some fishes in the Yangtze River, for example, *Zacco platypus* (Perdices, Cunha, & Coelho, 2004), *Leiocassis longirostris* (Xiao, Xia, & Ma, 2012; Yang, Xiao, Yu, & Xu, 2012), *Leptobotia elongata* (Tian, Duan, Wang, Liu, & Chen, 2013), *Siniperca chuatsi* (Tian et al., 2015), and *Saurogobio dabryi* (Li, Tang, Yu, & Liu, 2016). Compared to these fishes, the genetic diversity of *G. filifer* is currently high. Although the $F_{ST}$ test did not detect genetic differentiation among the sampled populations, the pairwise analysis (Table 3) revealed moderate divergence between the YHJ population and the others (except YYB, with the minimum value; Wright, 1978). However, the river distance from YHJ to YYB, which is about 195 km, is longer than that to YJJ (about 105 km) and there are not flow barriers between these populations. It was irrational and could be a sampling error—for sampling, time from YHJ was a week, however one to several months from other sampling sites. The XXJ population also displayed moderate differentiation from the four populations in the upper reaches of the Yangtze River (Table 3) and had the highest haplotype diversity and nucleotide diversity. This population was isolated with the others by Dongting Lake and may have less exchange with other population, especially the upstream populations. However, considering the small sample size, whether the results hold true for YHJ and XXJ is still unresolved.

Two dams were constructed in Yichang City (Figure 1) in 1981 (Gezhouba Dam) and 2003 (Three Gorges Dam), and they hindered the gene flow of populations between the upper and middle reaches of the Yangtze River. We did not detect genetic differentiation among populations within groups from the upper and middle reaches (Table 2), suggesting that the dams had minimal effects on the population genetics. However, on a large scale, the genetic diversity of populations in the middle reaches was higher than that of populations in the upper reaches, and the numbers of haplotypes, site variants, and unique haplotypes in populations from middle reaches were greater than that from upper reaches. It may be the result of unidirectional gene flows that the fish could migrate from upstream to downstream across dam rather than vice versa. Such hypothesis

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**TABLE 2** Analysis of molecular variance (AMOVA) among *Gobiobotia filifer* populations

| Source of variation | df | Sum of squares | Variance component | Percentage of variation |
|---------------------|----|----------------|--------------------|------------------------|
| The whole sample    | 7  | 7.97           | 0.02               | 4.55                   |
| Among populations   | 7  | 284            | 122.03             | 95.45                  |
| Within populations  | 284| 122.03         | 0.43               | 95.45                  |

**F$_{ST}$**: 0.0455

**F$_{SC}$**: 0.0349

**F$_{ST}$**: 0.0557

**F$_{CT}$**: 0.0215

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**TABLE 3** The $F_{ST}$ values among *Gobiobotia filifer* populations

| YYB | YHJ | YJJ | CCS | YJZ | YJL | YHH | XXJ |
|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   |     |     |     |     |     |     |     |
| 2   | 0.0183 |     |     |     |     |     |     |
| 3   | 0.0522 | 0.1455** |     |     |     |     |     |
| 4   | 0.0454 | 0.1444** | −0.0105 |     |     |     |     |
| 5   | 0.0334 | 0.1210** | 0.0454 | 0.0365 |     |     |     |
| 6   | 0.0562 | 0.1469** | 0.0358 | 0.0315 | 0.0214 |     |     |
| 7   | 0.0375 | 0.1273** | 0.0318 | 0.0304 | −0.0129 | −0.0073 |     |
| 8   | 0.0673* | 0.1593** | 0.0830* | 0.0780* | 0.0218 | 0.0269 | 0.0152 |

*Significant level at $p < .05$.
**Extremely significant at $p < .01$. 

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was supported by migration rate estimation (Table 4). Historically, the Three Gorges river section of 193 km above Gezhouba Dam is narrow and very turbulent before the construction of the Three Gorges Dam and Gezhouba Dam. Such ecological condition is difficult for *G. filifer* to migrate from downstream to upstream, and dam construction has further hindered the upward migration. Therefore, ecological barriers and dam should promote the existence of higher genetic diversity in downstream reaches than in the upper reaches.

### 4.2 | Sympatric population and evolutionary originally hypothesis

In this study, we found two distinct mtDNA haplotypes of *G. filifer* co-existing in all eight sampled sites, which reflects a so-called sympatric population. Genetically different populations of fishes are commonly detected between geographical stocks because of isolation or distant barriers (Yang, Tang, et al., 2012). Sympatric populations have also been documented in several freshwater fishes including perch, wall-eye, rainbow smelt, and several salmonid species (Bergek & Björklund, 2007; Dupont, Bournet, & Bernatchez, 2007; Ferguson & Taggart, 1991; Lu & Bernatchez, 1999; Østbye, Næsje, Bernatchez, Sandlund, & Hindar, 2005; Pålme, Laikre, & Ryman, 2013; Pigeon, Dodson, & Bernatchez, 1998; Wilson et al., 2004). Several hypotheses have been proposed to interpret such results. First, divergent genetic lineages may reflect cryptic species with the absence of morphological diagnostic characteristics for identification. The neotropical skipper butterfly *Astraptes fulgerator* is the best example (Hebert et al., 2004). Other animals, such as the bumble bee (Scriven, Whitehorn, Goulson, & Tinsley, 2016), mollusk (Sun et al., 2016), frog (Stuart, Inger, & Taylor, 2012), and fish (Borsa, Hisao, Carpenter, & Chen, 2013; Feulner, Kirschbaum, Schugardt, Ketmaier, & Tiedemann, 2006; Rossier, 2015), were also revealed to contain sympatric cryptic species as detected through mtDNA sequence analysis. The COI sequence is usually used as DNA barcoding for species identification with the criterion of 2% or 3% sequence divergence (e.g., Costa et al., 2007; Hebert et al., 2004; Loh, Bond, Ashton, Roberts, & Tibbetts, 2014; Shen, Guan, Wang, & Gan, 2016; Ward et al., 2005). Using this sequence and the criterion, all sampled Cyprinidae fish in the midstream of the Yangtze River were successfully identified (Shen et al., 2016). However, divergent levels at 2% or 3% do not indicate valid species (April, Mayden, Hanner, & Bernatchez, 2011; Hubert et al., 2008). In *G. filifer*, the greatest genetic distance for K2P was 2.2% between haplotypes 8 and 10 of COI (Table S4), which were lower than those among species in the genus (Yang, He, Freyhof, Kai, & Liu, 2006). Therefore, it cannot be inferred that there is a cryptic species in this fish.

Second, sympatric intraspecific divergences in mtDNA could be shaped by long-term isolation coupled to food niche and/or reproductive separation (Wimberger, 1994). Such genetic structuring is typically detected with phenotypic differences. Trophic and genetically separate sympatric populations have been reported in salmonid fishes such as Arctic char, brown trout, and whitefish inhabiting the postglacial lakes in the Northern Hemisphere, which are landlocked populations (e.g., Ferguson & Mason, 1981; Gowell, Quinn, & Taylor, 2012; May-McNally, Quinn, Woods, & Taylor, 2015; Power, Power, Reist, & Bajno, 2009; Præbel et al., 2013; Siwertsson et al., 2013). The food content of *G. filifer* has been examined, and most of its food sources are benthic organisms, such as mosquito larvae, *Limnoperma fortunei*, and aquatic insects (Wu et al., 2008). No distinct differences in food niche or morphological characteristics were found in the *G. filifer* population.

Third, distinct lineages in the same site can also be mixed populations from geographical subpopulations through invasion or introduction. Correlations between admixture genetic lineages and invasion events have been observed in sculpins *Cottus* spp. (Nolte, Freyhof, Stemhorn, & Tautz, 2005), guppies *Poecilia reticulata* (Lindholm et al., 2005), and zander *Sander lucioperca* (Eschbach et al., 2014). However, that might not be the case for *G. filifer*. Lineages were usually detected in invaded habitats, and the proportions of population size in each lineage were varied with the scales and plasticity of the introduced population (Eschbach et al., 2014; Nolte et al., 2005). However, no barriers hindered fish migration among the sampled sites in their long history until construction of the Gezhouba Dam and Three Gorges Dam, and we did not identify any documentation about fish introduction. In fact, there is little commercial or ecological interest in introducing *G. filifer*.

### Table 4 Posterior distribution of migration rate between *Gobiobota filifer* populations in upper and middle reaches using Bayesian analysis

| Parameter | 2.5% | 25.0% | Mode | 75.0% | 97.5% | Median | Mean |
|-----------|------|-------|------|-------|-------|--------|------|
| Θ<sub>UPG</sub> | 0.00493 | 0.00700 | 0.00830 | 0.00967 | 0.01247 | 0.00857 | 0.00862 |
| Θ<sub>MPG</sub> | 0.01220 | 0.01793 | 0.02183 | 0.02627 | 0.03807 | 0.02337 | 0.02427 |
| M<sub>MPG→UPG</sub> | 0 | 0 | 4.3 | 64.0 | 248.7 | 64.3 | 86.8 |
| M<sub>UPG→MPG</sub> | 650.7 | 889.3 | 980.3 | 996.7 | 1,000.0 | 894.3 | 869.7 |

θ, effective population size; M, immigration rate.

### Table 5 Mismatch analyses based on mtDNA Cyt b sequences for HapGroup 1 and HapGroup 2 and estimates of population expansion time

| Populations | r (95% intervals) | SSD (p-value) | HRag (p-value) | T (1,000 years ago) |
|-------------|-------------------|----------------|----------------|---------------------|
| HapGroup 1  | 2.195 (0.930, 5.832) | 0.0062 (.35) | 0.0390 (.5) | 14.3 (6.1, 38.0) |
| HapGroup 2  | 2.537 (1.268, 3.164) | 0.0051 (.05) | 0.0416 (.45) | 16.5 (8.3, 20.6) |
Fourth, divergent populations could have originated from secondary contacts of two distinct glacial refugia. Geographical isolation with secondary contacts would predict a high level of divergence between the mtDNA haplotypes (Grant & Bowen, 1998). There are inferred examples of secondary contacts in sympatric fish populations including _Pagellus erythrinus_ in the central Mediterranean Sea (Angiuli, Sola, Ardizzone, Fassatouli, & Rossi, 2016), _Rhinchithys cataractae_ in the rivers of southeastern British Columbia, Canada (Ruskey & Taylor, 2016), and _Salmo trutta_ in two tiny subarctic Swedish lakes (Andersson et al., 2017) and in the Loch Maree catchment, Scotland (Jacobs, Hughes, Robinson, Adams, & Elmer, 2018). This may be the most likely hypothesis for _G. filifer_. Global homogeneity implied that the two distinct populations came in contact postglacially at one position and then colonized novel environments synchronously. Population expansions (Table 5) occurred in the last glacial period (10,000–70,000 years ago), and temperature and rainfall began to increase at that time in east China (Song, Yu, & Zhu, 1998). Many fishes in the Yangtze River have been reported to experience population bottleneck followed by expansion, such as _Parabramis pekinensis_, _Squalidus argentatus_, _Gymnocypris dobulai_ (Yang, Xiao, et al., 2012), _Angiulli, Sola, Ardizzone, Fassatouli, & Rossi, 2016_), _G. filifer_ (Chen, Y. Y. (1998). _Biochemical Systematics and Ecology_, 152–160. https://doi.org/10.1016/S0369-801X(98)00003-8), _Adams, & Elmer, 2018_). This may be the most likely hypothesis for _G. filifer_. Global homogeneity implied that the two distinct populations came in contact postglacially at one position and then colonized novel environments synchronously. Population expansions (Table 5) occurred in the last glacial period (10,000–70,000 years ago), and temperature and rainfall began to increase at that time in east China (Song, Yu, & Zhu, 1998). Many fishes in the Yangtze River have been reported to experience population bottleneck followed by expansion, such as _Leiocassis longirostris_ (Yang, Xiao, et al., 2012), _Squalidus argentatus_ (Yang, Tang, et al., 2012), _Gymnocypris dobulai_ (Chan, Li, Hu, Liu, & Xu, 2016), and _Parabramis pekinensis_ (Chen et al., 2016). The asymmetric proportion of HapGroup 1 to HapGroup 2 populations could be interpreted as different founder sizes and panmixia after recontact of distinct populations, but nuclear maker is needed to support this hypothesis.

### 4.3 Concluding remarks

To our knowledge, this is the first report about sympatric genetically populations of fish in the Yangtze River. These fishes are not a cryptic species, but instead represent a secondary contact of distinct glacial refugia. This study highlights that historical and ecological factors play an important role in population patterns for fishes in the Yangtze River. _G. filifer_ is likely not the only example of a sympatric population in the Yangtze River.

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## CONFLICT OF INTEREST

None declared.

## AUTHORS CONTRIBUTIONS

Wang DQ, Gao L, Tian HW, Dong WW, and Duan XB collected samples. Wang DQ, Dong WW, and Duan XB performed the experiments and analyzed the data. Wang DQ, Liu SP, and Chen DQ prepared the data and wrote the manuscript. All authors read and approved the final manuscript.

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## DATA AVAILABILITY STATEMENT

All DNA sequences can be accessed via GenBank accessions: MK050193-MK050258 and MK834299-MK834309.

## REFERENCES

Andersson, A., Jansson, E., Wennerström, L., Chiriboga, F., Arnyasi, M., Kent, M. P., … Laikre, L. (2017). Complex genetic diversity patterns of cryptic, sympatric brown trout (_Salmo trutta_) populations in tiny mountain lakes. _Conservation Genetics_, 18, 1–15. https://doi.org/10.1007/s10592-017-0972-4

Angiuli, E., Sola, L., Ardizzone, G., Fassatouli, C., & Rossi, A. R. (2016). Phylogeography of the common pandora _Pagellus erythrinus_ in the central Mediterranean sea: Sympatric mitochondrial lineages and genetic homogeneity. _Marine Biology Research_, 12, 4–15.

April, J., Mayden, R. L., Hanner, R. H., & Bernatchez, L. (2011). Genetic calibration of species diversity among North America’s freshwater fishes. _Proceedings of the National Academy of Sciences of the United States of America_, 108, 10602–10607. https://doi.org/10.1073/pnas.1016437108

Beerli, P. (2006). Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. _Bioinformatics_, 22, 341–345. https://doi.org/10.1093/bioinformatics/bti803

Bergek, S., & Björklund, M. (2007). Cryptic barriers to dispersal within a lake allow genetic differentiation of Eurasian perch. _Evolution_, 61, 2035–2041. https://doi.org/10.1111/j.1558-5646.2007.00163.x

Borsa, P., Hsiao, D. R., Carpenter, K. E., & Chen, W. J. (2013). Cranial morphometrics and mitochondrial dna sequences distinguish cryptic species of the longface emperor (_Lethrinus olivaceus_), an emblematic fish of Indo-west pacific coral reefs. _Comptes Rendus - Biologies_, 336, 505–514.

Chan, J., Li, W., Hu, X., Liu, Y., & Xu, Q. (2016). Genetic diversity and population structure analysis of jinghai-tibetan plateau schizothoracine fish (_Gymnocypris dobulai_ based on mtDNA d-loop sequences. _Biochemical Systematics and Ecology_, 69, 152–160. https://doi.org/10.1016/j.bse.2016.09.004

Chen, H. J., Wang, D. Q., Duan, X. B., Chen, D. Q., Liu, S. P., & Li, Y. (2016). Genetic diversity of white bream, _Parabramis pekinensis_ from the middle Yangtze River. _Chinese Journal of Ecology_, 35, 2175–2181.

Chen, Y. Y. (1998). _Fauna sinica. Osteichthyes. Cypriniformes II_ (pp. 389–413). Beijing, China: Science Press.

Costa, F. O., deWaard, J. R., Bouthillier, J., Ratnasingham, S., Dooh, R. T., Hajibabaei, M., & Hebert, P. D. N. (2007). Biological identifications through DNA barcodes: The case of the Crustacea. _Canadian Journal of Fisheries and Aquatic Science_, 64, 272–295. https://doi.org/10.1139/f07-008

Dupont, P. P., Bourret, V., & Bernatchez, L. (2007). Interplay between ecological, behavioural and historical factors in shaping the genetic structure of sympatric walleye populations (_Sander vitreus_). _Molecular Ecology_, 16, 937–951. https://doi.org/10.1111/j.1365-294X.2006.03205.x

Dynesius, M., & Jansson, R. (2000). Evolutionary consequences of changes in species’ geographical distributions driven by Milankovitch climate oscillations. _Proceedings of the National Academy of Sciences of the United States of America_, 97, 9115–9120. https://doi.org/10.1073/pnas.97.18.9115

Eschbach, E., Nolte, A. W., Kohnhalm, K., Kersten, P., Kail, J., & Arlinghaus, R. (2014). Population differentiation of zander (_Sander lucioperca_) across native and newly colonized ranges suggests increasing admixture in the course of an invasion. _Evolutionary Applications_, 7, 555–568.
Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.

Fan, Z. H., Ba, J. W., & Duan, X. B. (2012). Studies on fish resources and species diversity in the middle reaches of the Yangtze River from Yichang to Chenglingji section. *Freshwater Fisheries*, 42, 20–25.

Ferguson, A., & Mason, F. M. (1981). Allozyme evidence for reproductively isolated sympatric populations of brown trout *Salmo trutta* L. in Lough Melvin, Ireland. *Journal of Fish Biology*, 18, 629–642. https://doi.org/10.1111/j.1095-8649.1981.tb03805.x

Ferguson, A., & Taggart, J. (1991). Genetic differentiation among the sympatric brown trout (Salmo trutta) populations of Lough Melvin, Ireland. *Biological Journal of the Linnean Society*, 43, 221–237. https://doi.org/10.1111/j.1095-8312.1991.tb00595.x

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.

Feulner, P. G., Kirschbaum, F., Schugardt, C., Ketmaier, V., & Tiedemann, J. (2016). *Platyrrhinus cataphractus* in the St. Lawrence River estuary, Quebec, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1739–1747.
Shen, Y., Guan, L., Wang, D., & Gan, X. (2016). DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. *Ecology and Evolution*, 6, 2702–2713. https://doi.org/10.1002/ece3.2060

Siwertsson, A., Knudsen, R., Præbel, K., Adams, C. E., Newton, J., & Stuart, B. L., Inger, R. F., & Voris, H. K. (2006). High level of cryptic speciation in the longsnout catfish *Leiocassis longirostris*, in the Yangtze River revealed using mitochondrial DNA cytochrome b sequences. *Acta Zoologica Sinica*, 32, 305–313. https://doi.org/10.1016/j.ajas.2012.09.002

Xiao, F., Li, H. Y., Duan, X. B., Liu, S. P., & Chen, D. Q. (2015). Present status of fishery resources in Yibin section of the upper Yangtze River. *Journal of Southwestern University (Natural Science Edition)*, 37, 43–50.

Yang, G., Xiao, M., Yu, Y., & Xu, S. (2012). Genetic variation at mtDNA and microsatellite loci in Chinese longsnout catfish (*Leiocassis longirostris*). *Molecular Biology Reports*, 39, 4605–4617. https://doi.org/10.1007/s11033-011-1252-x

Yang, J., He, S., Freyhof, J., Kai, W., & Liu, H. (2006). The phylogenetic relationships of the Gobioninae (Teleostei: Cyprinidae) inferred from mitochondrial cytochrome b gene sequences. *Hydrobiologia*, 553, 255–266. https://doi.org/10.1007/S10750-005-1301-3

Yang, J.-Q., Tang, W.-Q., Liao, T.-Y., Sun, Y., Zhou, Z.-C., Han, C.-C., ... Lin, H.-D. (2012). Phylogeographical analysis on *Squalidus argentatus* recapitulates historical landscapes and drainage evolution on the island of Taiwan and Mainland China. *International Journal of Molecular Sciences*, 13, 1405–1425. https://doi.org/10.3390/ijms13021405

Yang, S., Xin, G., Ma, B. S., Kong, Y. A., & Liu, H. (2010). Seasonal dynamics of fish community in Mudong section of the Three Gorges reservoir of the Yangtze River, China. *Chinese Journal of Applied and Environmental Biology*, 16, 555–560.

Zardoya, R., & Doadrio, I. (1999). Molecular evidence on the evolutionary and biogeographical patterns of European cyprinids. *Journal of Molecular Evolution*, 49, 227–237. https://doi.org/10.1007/PL00006545

Zhou, W., Song, N., Wang, J., Jie, Z., & Gao, T. (2016). Effects of geological changes and climatic fluctuations on the demographic histories and low genetic diversity of *Squaliobarbus curriculus* in Yellow River. *Gene*, 590, 149–158. https://doi.org/10.1016/j.gene.2016.06.009

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.