Morphological Variation Among Wild Populations of Chinese Rare Minnow (*Gobiocypris rarus*): Deciphering the Role of Evolutionary Processes

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Morphological Variation Among Wild Populations of Chinese Rare Minnow (*Gobiocypris rarus*): Deciphering the Role of Evolutionary Processes

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***Gobiocypris rarus* Ye et Fu (1983) is an endemic cyprinid fish in China, and is considered to be an endangered species. From a conservation perspective, its population structure is interesting. In the present study, morphological variation, including morphometric and meristic traits, was assessed among wild samples collected in the upper Yangtze River basin. There were no significant meristic differences between sexes or among populations, except for scales in lateral line (LS). However, there were significant morphometric differences not only between sexes but also among populations. In discriminant function analysis, the first four discriminant functions explained 75.5% and 78% of the among-population morphometric variation for males and females, respectively. Almost all the truss network morphometric traits significantly contributed to population discrimination. By using all of the truss network morphometric traits, overall random assignments of male and female individuals into their original population were both 73.5%. In addition, the degree of differentiation in phenotypic traits (P_{ST}) significantly exceeds that in neutral molecular markers (F_{ST}). However, no significant correlation between P_{ST} and F_{ST} was found in males or females. Overall, these results suggest that two evolutionary processes, including phenotypic plasticity and natural selection, may contribute to the morphological patterns observed in *G. rarus*.**

Key words: *Gobiocypris rarus*, morphological variation, morphometrics, sexual dimorphism, population differentiation

INTRODUCTION

Studies of the population structure of threatened fishes are of theoretical interest for evolutionary biologists and of practical value for environmental managers. Quantitative variations of morphological and genetic traits have been extensively used to describe the population structure in many organisms (Murta, 2000; Silva, 2003; Turan et al., 2006; Clabaut et al., 2007), and have been of great concern for improving the conservation of imperiled species (Frankham et al., 2002).

In wild populations, adaptation to a local environment is often revealed by the analysis of morphological traits (Langerhans et al., 2003; Langerhans et al., 2007; Aguirre et al., 2008; Páez et al., 2008; Kristjánsson et al., 2011). Variation in morphological traits may result from three main evolutionary processes: genetic drift, natural selection, and/ or phenotypic plasticity. The latter case refers to cases in which a genotype adapts plastically to its environment, mainly through ontogenetic changes (Roff, 1992; Gianoli

and Valladares, 2012). Whether patterns of morphological adaptation are due to phenotypic plasticity or natural selection, both imply a strong role for the environment (either biotic or abiotic). However, the consequences for the evolutionary and ecological dynamics of populations are not the same, as in one case (natural selection) variation is transmitted to the next generation, whereas in the other case (phenotypic plasticity) variation is generally not transmitted (however, phenotypic plasticity can sometimes be transmitted, for instance through epigenetic or habitat inheritance, Agrawal, 2001; Pigliucci, 2005; Danchin et al., 2011). In contrast, genetic drift does not imply environmental effects, but rather indicates that effective population size is an important parameter to consider for predicting the evolutionary dynamics of populations. Deciphering between these evolutionary processes is a difficult but important task for setting appropriate conservation plans. This is particularly challenging in aquatic ecosystems, such as streams or rivers, because these ecosystems exhibit great spatial or temporal variability in both abiotic and biotic habitat parameters (Lowe-McConnell, 1987; Langerhans et al., 2007; Aguirre, 2009; Elmer et al., 2010; Collin and Fumagalli, 2011), and because gene flow and movements are constrained not only by river basin boundaries, but also by the upstream-downstream flows that often lead to asymmetric gene flow, hence dampening local

adaptation in downstream sites (Kawecki and Holt, 2002; Morrissey and Kerckhove, 2009; Stelkens et al., 2012).

Sexual dimorphism is also a frequent source of morphological variation among populations (Kitano et al., 2007; Williams and Carroll, 2009). Differentiation in external structures between males and females are usually not directly related to reproduction. These sexually dimorphic traits in external morphology can result from both sexual and natural selection (Andersson, 1994; Oliveira and Almada, 1995; Kitano et al., 2007). Knowledge of sexual dimorphism is indispensable in the study of population structure on morphological traits, notably because a biased sex ratio can lead to spatial patterns for which processes can be incorrectly inferred if sexual dimorphism is not accounted for (Oliveira and Almada, 1995; Skjæraasen et al., 2006; Kitano et al., 2007; Aguirre et al., 2008).

The rare minnow, *Gobiocypris rarus* Ye et Fu (1983), is an endemic cyprinid fish in China (Ye and Fu, 1983; Chen, 1998). It is considered to be an endangered species due to its narrow distribution and small number of large remnant populations (Le and Chen, 1998; Wang et al., 1998; Li et al., 2004; Wang and Xie, 2004; Xiong et al., 2009). Its distribution is limited to the western part of Sichuan Province, China (Ding, 1994; Wang and Cao, 1997; Chen, 1998; Le and Chen, 1998). All known habitats of rare minnow are scattered, separated by dozens to hundreds of miles, indicating a discontinuous distribution for the species. Meanwhile, this fish has been used extensively as an aquatic laboratory animal in toxicology, fish pathology, developmental biology, and genetics (Wang, 1992, 1996, 1999; Wang et al., 1994; Wang and Cao, 1997; Jia et al., 2002; Zhong et al., 2005; Pei et al., 2008; Su et al., 2008). Very little is known about the adaptation of this species to environmental factors, which limits the setting of appropriate conservation plans (but see Shao et al., 2007; He et al., 2012).

The main goals of our study are to investigate intra- and inter-population morphological variation of *G. rarus* and to test for biological, ecological, and evolutionary processes driving morphological variation. To do so, we explored patterns of variation in morphometric and meristic characters of *G. rarus* in four sub-basins of the upper Yangtze River basin, and first tested whether this fish species displays sexual dimorphism in external morphology. We next compared the degree of phenotypic differentiation measured by the P_{ST} index, analogous to Q_{ST}, but affected by environmental effects (Spitze, 1993; Raeymaekers et al., 2007; Brommer, 2011), against the neutral expectation set by allelic divergence in microsatellite markers (F_{ST}) to assess the relative roles of genetic drift, natural selection, and phenotypic plasticity for the observed population differentiations in body shape.

Several studies revealed that the accuracy of the approximation of Q_{ST} by P_{ST} is not dependent on the heritability of the trait, but rather depends on the extent to which variance across populations relative to variance within populations are determined by additive genetic effects (Brommer, 2011). It is well understood that nonadditive genetic variance or environmental factors and genotype-environment interactions may distort additive genetic variance when investigating only phenotypic variances (Pujol et al., 2008). When doing P_{ST}/F_{ST} comparisons, it is usually assumed first that phenotypic variance equals additive genetic variance, excluding nonadditive genetic and environmental effects (Merilä, 1997). If P_{ST} is not significantly different from F_{ST}, the divergence in quantitative traits is likely to be due to genetic drift. However, if P_{ST} is significantly different from F_{ST} (either greater or smaller), selection or phenotypic plasticity are likely factors underlying divergence in quantitative traits (Merilä and Crnokrak, 2001).

MATERIALS AND METHODS

Sample collection

G. rarus were collected by nets in 2008 from nine sites, located in the Sichuan Province, China (Fig. 1, Table 1). These nine sites are from four river basins: the Tuojiang River, the Minjiang River, the Dadu River, and the Qingyi River. Following capture, fish were placed in water vats to keep them alive during transportation. They were then anesthetized by MS222 before they were weighed and measured. Finally, fish were dissected in order to determine the sex. Sample size in this study varied between 30 and 50, whereas Reist (1985) recommended for morphological analysis the sample size of at least 25 specimens.
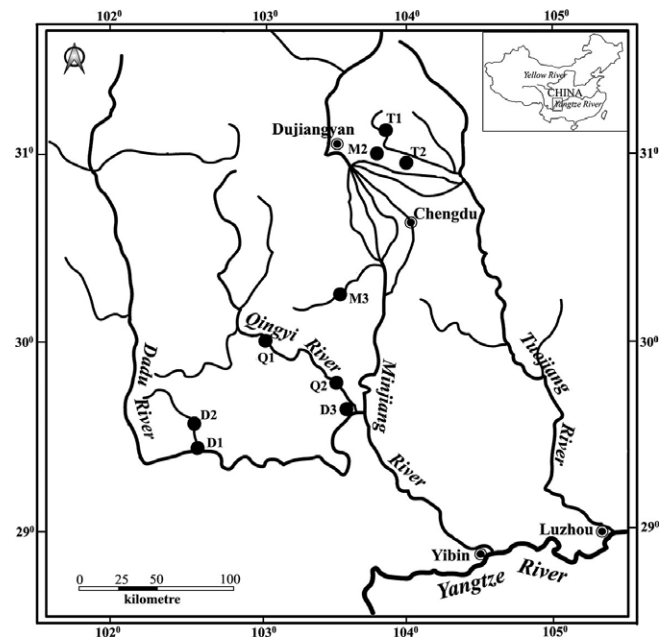

Fig. 1. Map of the sampling locations for *Gobiocypris rarus*.

Table 1. Sampling details of *Gobiocypris rarus* used in this study.

Sampling sites	Abbr.	Location	Sample size	Sex (M/F)	MSL
Haiwozi	T1	Tuojiang River basin	50	27/23	44.40 (6.36)
Penzhou City	T2	Tuojiang River basin	50	17/33	34.81 (6.10)
Lichun Town	M2	Minjiang River basin	30	18/12	38.00 (6.71)
Qionglai City	M3	Minjiang River basin	50	17/33	38.40 (3.76)
Yaan City	Q1	Qingyi River basin	50	23/27	37.15 (5.75)
Jiajiang County	Q2	Qingyi River basin	50	40/10	31.65 (5.26)
Liusha River mouth	D1	Dadu River basin	31	23/8	37.17 (6.35)
Jiuxiang Town	D2	Dadu River basin	50	16/34	34.21 (4.56)
Leshan City	D3	Dadu River basin	50	25/25	41.50 (4.68)

Abbr. = Abbreviation. MSL represents mean standard length (mm) of each site. Standard deviations of MSL are given in parentheses.
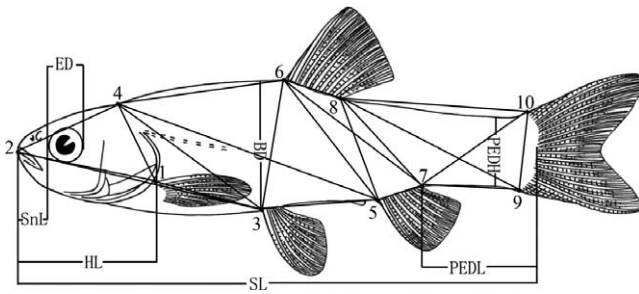
Fig. 2. Traditional morphometric measurements and truss network measurements based on 10 landmarks of *Gobiocypris rarus* are shown. The 10 landmarks represent: 1, pectoral fin insertion; 2, anteriormost tip of snout; 3, pelvic fin insertion; 4, posterior point of the neurocranium; 5, anal fin origin; 6, dorsal fin origin; 7, posterior end of anal fin base; 8, posterior end of dorsal fin base; 9, ventral origin of caudal fin; 10, dorsal origin of caudal fin.

Morphometrics

All measurements were taken on the left side of fish by the same person in order to minimize measurement bias. Traditional measurements and the truss network system were used to describe the shape of fish body, based on the methods of Strauss and Bookstein (1982) and Bookstein et al. (1985). Traditional data, such as standard length (SL), body depth (BD), head length (HL), snout length (SnL), eye diameter (ED), distance between eyes (DBE), peduncle length (PEDL), and peduncle height (PEDH) were recorded, as shown in Fig. 2. In the truss network analysis, 10 landmarks determining 21 distances were produced and measured, as illustrated in Fig. 2. The truss data are expressed as D1-2, D2-4, D4-3, and so on. For example, D1-2 means the distance between landmarks 1 and 2. Images of thawed fish were acquired from a fixed distance with a high-resolution digital camera. DBE was manually measured by using a digital caliper with an accuracy of 0.01 mm, and other measurements were analyzed using TpsDig 2.04 (Rohlf, 2005).

Meristics

Meristic characters were examined using the number of: pectoral fin rays (PFR), dorsal fin rays (DFR), ventral fin rays (VFR), anal fin rays (AFR), and scales in lateral line (LS) under a binocular stereomicroscope.

Statistical analysis

Morphometric and meristic characters were considered separately in statistical analyses, as these variables are different both statistically (the former are continuous while the later are discrete) and biologically (the latter are fixed early in development, while the former are more susceptible to the environment) (Ihssen et al., 1981).

Transformation of absolute measurements to size-independent shape variables was the first step of the analysis. In the present study, no significant correlations were observed between meristic characters and standard lengths of samples, indicating meristic characters were independent of fish size and the original meristic data were not transformed. Difference in meristic characters between male and female samples was revealed by non-parametric Kruskal-Wallis test. To identify any statistically significant differences in meristic characters among populations, non-parametric Kruskal-Wallis tests were also performed. After Kruskall-Wallis test, multiple comparison tests between populations were conducted using the 'pgirmess' package in the R software (Ihaka and Gentleman, 1996; Giraudoux, 2006).

Significant correlations were observed between morphometric characters and standard length of samples. In order to eliminate

any variation resulting from allometric growth, all morphometric measurements were standardized according to Reist (1985). The formula is $M_{adj} = \log M - b (\log L_o - \log L_s)$, where M_{adj} is the size adjusted measurement, M the original morphometric measurement, L_s the overall mean of standard length for all fish from all samples, and L_o the standard length of fish. The parameter b was estimated for each character from the observed data as the slope of the regression of $\log M$ on $\log L_o$, using all specimens. Correlation coefficients between transformed variables and standard length were calculated to check if the data transformation was effective in removing the effect of size in the data.

The homoscedasticity of morphometric data was firstly tested by using Bartlett's test (Snedecor and Cochran, 1989) and their normality by using Shapiro's test (Shapiro and Wilk, 1965). There were no severe departures from homoscedasticity and normality ($P > 0.05$). Following size-correction, the differences in morphometric variables between male and female samples were determined using multivariate analysis of variance (MANOVA). If significant sex differences are identified, male and female samples should be analyzed separately. To verify if there were any statistically significant differences among populations, both MANOVA and analysis of variance (ANOVA) were performed. Afterwards, discriminant analysis (DA) was conducted to determine which morphometric variables best discriminate populations. Standardized coefficients for each variable in each discriminant function represent the contribution of the respective variable to the discrimination among populations. A random Monte Carlo test with 1000 permutations was used to test the significance of morphometric variables among populations. A holdout procedure (i.e., 2/3 of the data as the training set and the remaining 1/3 as the test set) was performed to test the ability of the model to discriminate between populations, so that the proportion of individuals correctly re-allocated was obtained. All these analyses were conducted using R software (Ihaka and Gentleman, 1996). In MANOVA and discriminant analysis, only truss network morphometric traits were used.

Correlating morphology and genetics

Pairwise genetic divergence at neutral markers was measured as F_{ST} values obtained from eight microsatellite markers (see He et al., 2012). The specimens used in microsatellite analyses were the same as those in the present study (Table 1). In order to correspond with the morphological analysis, we recalculated F_{ST} values by separating female and male samples. The riparian geographical distance (RD) was calculated along the closest connected water system in Google Earth 5.0 (Google Inc., 2009).

A dimensionless measure of among-population phenotypic differentiation for quantitative traits, analogous to Wright's (1951) F_{ST}, can be defined as $P_{ST} = c \sigma_b^2/(c \sigma_b^2 + 2h^2 \sigma_w^2)$ (Spitze, 1993; Storz, 2002; Raeymaekers et al., 2007; Brommer, 2011). In this formula, σ_b^2 and σ_w^2 are the inter-population and intra-population variance components for a phenotypic trait in the wild, respectively; h^2 denotes the heritability (the proportion of phenotypic variance that is because of additive genetic effects); the scalar c expresses the proportion of the total variance that is presumed to be because of additive genetic variance across populations. Due to the inability to estimate true c and h^2 for a particular trait across wild populations, we assumed that $c = h^2$ in the present study. Similarly, female and male samples were separated before calculating P_{ST} values. The partitioning of phenotypic variance within and among populations of *G. rarus* was assessed using ANOVA. A intra-population variance (σ_w^2) was estimated by equating observed intra-population mean squares (MS_{within}) to their expectations. The added variance component attributable to differences among populations (σ_b^2) was estimated as $\sigma_b^2 = (MS_{between} - MS_{within})/n_0$, where $MS_{between}$ is an unbiased estimate of the between-population variance and n_0 is the average sample size (Sokal and Rohlf, 1995). For each comparison, the average sample size n_0 was calculated as $n_0 = 1/(\alpha - 1)*$

$(\sum_{i}^{\alpha} n_i - \sum_{i}^{\alpha} n_i^2 / \sum_{i}^{\alpha} n_i)$, where α equals the number of populations compared and n equals to the number of individuals in the ith population sample. CIs for P_{ST} were estimated by 1000 bootstrap replicates. P_{ST} at each trait was estimated between all population pairs. These analyses were conducted using the R software.

To test whether levels of phenotypic divergence, genetic divergence, and riparian geographic distance were correlated, we calculated the correlations between pairwise P_{ST}, F_{ST} and riparian geographic distance with Mantel tests (Mantel, 1967). Partial Mantel tests were used to test correlations between pairwise P_{ST} and F_{ST} after controlling for riparian geographic distance. These analyses were conducted using the library *vegan* in the R software. In order to compare the level of phenotypic divergence with neutral genetic divergence, pairwise P_{ST} values were compared with pairwise F_{ST} values using Wilcoxon signed-rank test in STATISTICA 6.0 (StatSoft, 2001).

RESULTS

Meristics

No significant sex dimorphism was revealed in meristic characters ($P > 0.05$). The non-parametric Kruskal-Wallis tests also showed no significant differences among populations for almost all meristic characters ($P > 0.05$) except for scales in lateral line (LS). The multiple comparison tests revealed significant LS differences only between some population pairs ($P < 0.05$, Fig. 3). Overall, the ranges of all meristic counts widely overlapped, and the modes of meristic characters were equal or close to each other among populations of *G. rarus*.

Morphometrics

After size-adjustment calculations, no significant correlation coefficients between transformed variables and standard length were found, indicating that the size effect had been successfully removed with the allometric transformation. Therefore, none of the variables was discarded from the following analyses.

Overall, there were significant differences between
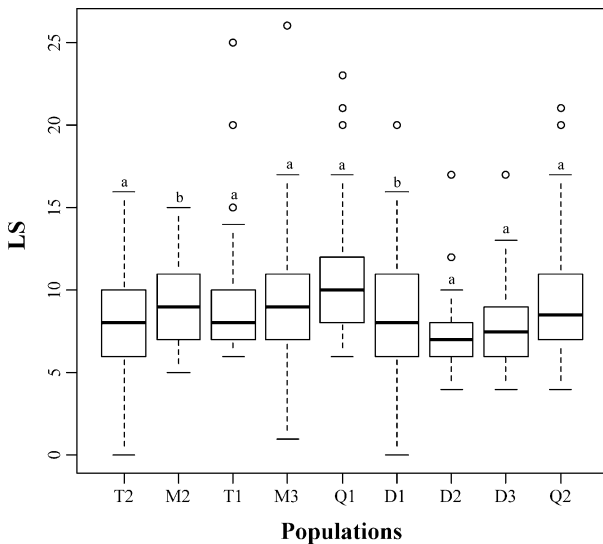
Fig. 3. The box-plot of a significant meristic character LS (scales in lateral line) of *Gobiocypris rarus* in each population: median, 25–75%, maximum, minimum, outlier. Different characters (a/b) marked in the figure represent significant differences, while the same character means non-significant difference.

males and females when twenty-one truss network morphometric variables were considered in a single analysis (MANOVA, $P < 0.001$). ANOVA revealed that eighteen morphometric traits (BD, DBE, PEDL, PEDH, D1_2, D4_3, D3_1, D2_3, D4_6, D5_3, D3_6, D4_5, D6_8, D7_5, D10_9, D9_7, D7_10, and D8_9) showed significant differences between sexes ($P < 0.05$). Therefore, male and female samples were separately analyzed in further analyses.

For both female and male samples, MANOVA showed that morphological differences among populations were significant for all truss network morphometric measurements ($P < 0.001$). ANOVA showed that most of all the morphometric traits in females were significantly different among populations, with the same result for males (Table 2).

In discriminant function analysis, eight discriminant functions (DFs) were generated, and the random Monte Carlo permutation test showed that all studied populations were significantly discriminated not only in females but also in males ($P < 0.001$). The first four functions of the discriminant analysis explained 78.0% of variance in all the truss network morphometric traits of *G. rarus* female samples, while explaining 75.5% of variance for male samples. Most of truss network morphometric traits in females (17 traits) or males (16 traits) had high allometric shape contributions to the first four discriminant functions. The truss network morphometric traits of females and males contributing to the first two discriminant functions are shown in Fig. 4 and Fig. 5, respectively.

After separating male and female samples, the overall random assignment of individuals into their original popula-

Table 2. Results (*F* value and *P* value) from ANOVA, degree of phenotypic divergence in quantitative traits among populations (P_{ST}) for each size-corrected morphometric character.

Variables	Female			Male		
	F value	*P* value	P_{ST}	*F* value	*P* value	P_{ST}
HL	133.610	<0.001	0.749	82.642	<0.001	0.644
DBE	116.320	<0.001	0.721	31.646	<0.001	0.404
D1_2	57.439	<0.001	0.559	1.713	0.192	0.016
D1_4	57.253	<0.001	0.558	3.217	0.074	0.047
D7_10	37.079	<0.001	0.448	63.560	<0.001	0.580
SnL	29.776	<0.001	0.393	45.972	<0.001	0.499
ED	26.943	<0.001	0.368	18.297	<0.001	0.277
D3_1	25.266	<0.001	0.353	17.179	<0.001	0.263
PEDL	22.487	<0.001	0.326	23.544	<0.001	0.333
D10_9	20.768	<0.001	0.308	26.142	<0.001	0.357
D9_7	18.137	<0.001	0.278	25.786	<0.001	0.354
D6_8	16.087	<0.001	0.253	6.361	0.012	0.106
D2_4	14.015	<0.001	0.226	3.126	0.079	0.045
D8_10	6.431	0.012	0.109	19.740	<0.001	0.293
D8_9	4.430	0.037	0.072	11.422	<0.001	0.187
D5_3	3.979	0.047	0.063	22.543	<0.001	0.323
D6_5	3.659	0.057	0.056	2.493	0.116	0.032
D3_6	3.092	0.080	0.045	6.297	0.013	0.105
PEDH	1.670	0.198	0.015	0.719	0.398	–0.006
D6_7	1.323	0.251	0.007	0.162	0.688	–0.019
D4_5	1.194	0.276	0.004	3.000	0.085	0.042
D4_6	0.838	0.361	–0.004	6.976	0.009	0.117
D8_7	0.767	0.382	–0.005	0.886	0.348	–0.003
D7_5	0.504	0.479	–0.011	3.033	0.083	0.043
D4_3	0.378	0.539	–0.014	3.322	0.070	0.049
D2_3	0.253	0.616	–0.017	7.615	0.006	0.128
D5_8	0.244	0.622	–0.017	0.520	0.472	–0.011
BD	0.020	0.887	–0.023	1.137	0.288	0.003
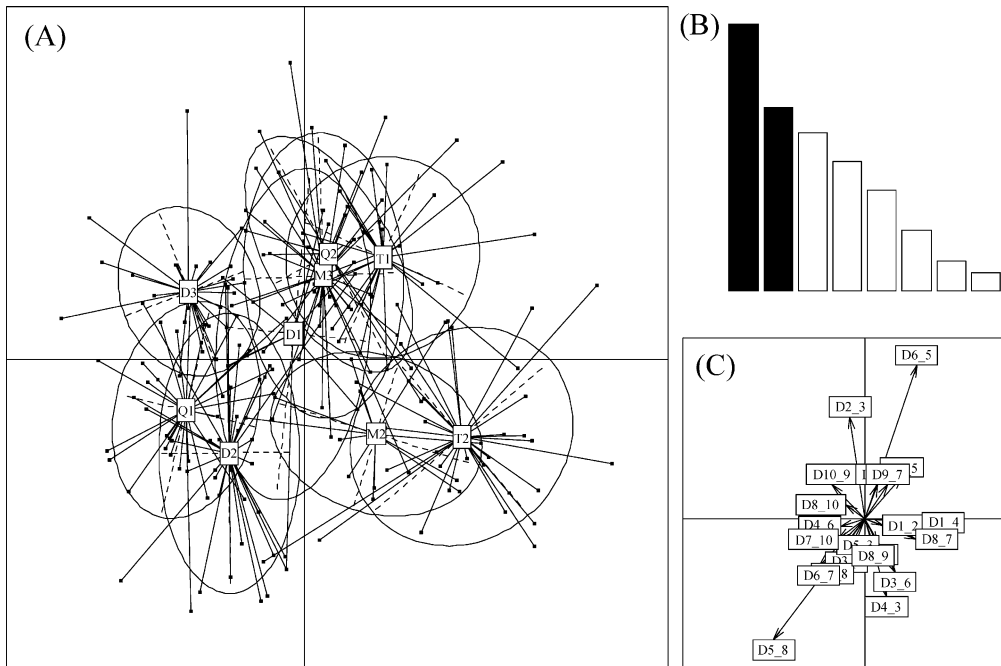
Fig. 4. Plots from discriminant analysis (DA) upon the standardized truss network morphometric data of female samples. **(A)** Axis 1 accounts for 28% and axis 2 for 19% of between-population variability, respectively. Each population is presented as ellipsoid with different numbers in the center; **(B)** Histogram showing eigenvalues of the DA; **(C)** Contribution of morphometric characters to the first and second discriminant functions.
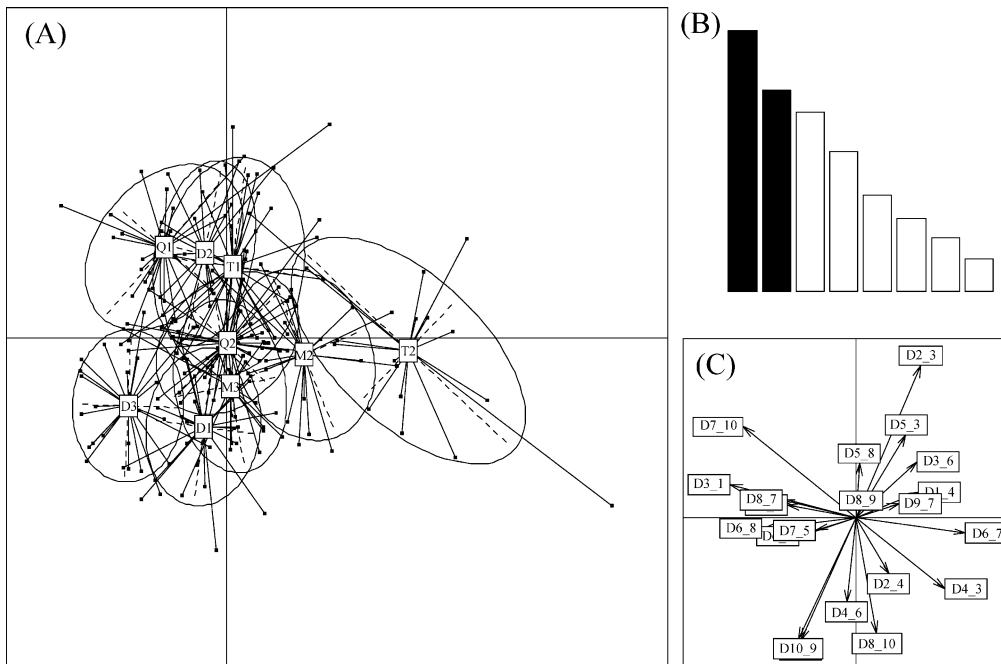
Fig. 5. Plots from discriminant analysis (DA) upon the standardized truss network morphometric data of male samples. **(A)** Axis 1 accounts for 25% and axis 2 for 19% of between-population variability, respectively. Each population is presented as ellipsoid with different numbers in the center; **(B)** Histogram showing eigenvalues of the DA; **(C)** Contribution of morphometric characters to the first and second discriminant functions.

100% of the Q2 female individuals were correctly classified to their original group. For male samples, almost all the populations had high correct classification (> 62.5%), except for population M3 (only 50%). The highest correct classification was found in population T2 (100%).

Comparison of genetic and morphological divergence

For females, phenotypic divergence (P_{ST}) among populations for each morphometric trait varied from –0.023 (BD) to 0.749 (HL), with a mean value of 0.208 (Table 2). For males, phenotypic divergence (P_{ST}) among populations for each morphometric trait varied from –0.019 (D6_7) to 0.644 (HL), with a mean value of 0.186 (Table 2). Pairwise mean P_{ST} comparison of populations for all morphometric traits varied from 0.026 (between populations M2 and T2) to 0.267 (between populations T1 and D2) for females, and from 0.006 (between populations M3 and D1) to 0.196 (between populations Q2 and T2) for males (Table 5).

For both female and male samples, Mantel tests revealed no significant correlations between mean P_{ST} and mean F_{ST}, irrespective of whether riparian geographic distance was considered as a controlled factor (Table 6). Pairwise Mantel tests also showed that no significant correlations between mean P_{ST} and riparian geographic distance for both female and male samples (Table 6). For male samples, no significant correlations between P_{ST} for single morphometric trait and F_{ST} for all neutral microsatellite loci were found ($P > 0.05$). Similarly, there were also no significant correlations between P_{ST} for most of single morphometric traits and F_{ST} for all neutral

tions were 73.5% for both females and males by hold-out procedure (Table 3 and Table 4). For female samples, most populations had relatively high correct classification (> 62.5%), except for population M2 (only 33.3%) and D1 (0%). Indeed,

Table 3. Proportion of female individuals classified correctly into their original population over 21 truss network morphometric characters using hold-out procedure.

Population	T1	T2	M2	M3	D1	D2	D3	Q1	Q2
T1	**0.750**	0.063	0.000	0.083	0.500	0.000	0.000	0.000	0.000
T2	0.250	**0.625**	0.333	0.000	0.000	0.000	0.000	0.000	0.000
M2	0.000	0.125	**0.333**	0.000	0.000	0.000	0.000	0.000	0.000
M3	0.000	0.063	0.000	**0.917**	0.000	0.000	0.100	0.000	0.000
D1	0.000	0.125	0.000	0.000	**0.000**	0.000	0.100	0.000	0.000
D2	0.000	0.000	0.000	0.000	0.000	**0.818**	0.000	0.250	0.000
D3	0.000	0.000	0.333	0.000	0.500	0.182	**0.800**	0.000	0.000
Q1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.750**	0.000
Q2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**1.000**

Table 4. Proportion of male individuals classified correctly into their original population over 21 truss network morphometric characters using hold-out procedure.

Population	T1	T2	M2	M3	D1	D2	D3	Q1	Q2
T1	**0.625**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
T2	0.000	**1.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
M2	0.000	0.000	**0.625**	0.167	0.111	0.000	0.000	0.000	0.000
M3	0.000	0.000	0.125	**0.500**	0.111	0.000	0.000	0.000	0.000
D1	0.250	0.000	0.000	0.000	**0.667**	0.000	0.000	0.000	0.000
D2	0.000	0.000	0.125	0.000	0.000	**0.833**	0.125	0.000	0.000
D3	0.000	0.000	0.000	0.000	0.111	0.000	**0.750**	0.000	0.000
Q1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.750**	0.067
Q2	0.125	0.000	0.125	0.333	0.000	0.167	0.125	0.250	**0.867**

Table 5. Pairwise P_{ST} values of wild populations of *G. rarus*, using all size-corrected morphometric variables. Values below diagonal are based on female samples, while values above diagonal are based on male samples.

Population	T1	T2	M2	M3	D1	D2	D3	Q1	Q2
T1	0.000	0.123	0.062	0.055	0.060	0.108	0.075	0.078	0.087
T2	0.103	0.000	0.042	0.066	0.121	0.166	0.191	0.187	0.196
M2	0.169	0.026	0.000	0.020	0.034	0.136	0.110	0.123	0.117
M3	0.129	0.086	0.103	0.000	0.006	0.114	0.069	0.096	0.121
D1	0.132	0.050	0.035	0.098	0.000	0.128	0.052	0.115	0.109
D2	0.267	0.136	0.092	0.190	0.109	0.000	0.089	0.037	0.138
D3	0.199	0.127	0.113	0.148	0.047	0.066	0.000	0.046	0.125
Q1	0.223	0.140	0.107	0.163	0.105	0.049	0.057	0.000	0.139
Q2	0.066	0.095	0.113	0.137	0.068	0.184	0.106	0.150	0.000

Table 6. Correlations (*r*) and significance (*P*) between P_{ST}, F_{ST} and riparian geographic distance by pairwise and partial Mantel test. When doing partial Mantel test, riparian geographic distance was considered as a controlled factor.

Comparisons	Female samples		Male samples	
	r	P-value	r	P-value
Pairwise Mantel test				
P_{ST}-F_{ST}	0.336	0.073	−0.137	0.747
P_{ST}-Riparian geographic distance	0.236	0.080	0.004	0.470
Partial Mantel test				
P_{ST}-F_{ST}	0.280	0.104	−0.166	0.790

microsatellite loci in female samples ($P > 0.05$). However, significant correlations between P_{ST} and F_{ST} were found in two single morphometric traits (D4_6 and D10_9) of female samples, irrespective of whether riparian geographic distance was controlled for (Fig. 6).

A comparison of F_{ST} and mean P_{ST} values between population pairs of *G. rarus* revealed that the degree of differentiation in phenotypic traits significantly exceeds that in neutral molecular markers by using Wilcoxon signed-rank tests (Females: $z = −4.666$, $P < 0.001$; Males: $z = −3.079$, $P = 0.002$).

Fig. 6. Significant relationship between pairwise F_{ST} values at eight neutral microsatellite loci and pairwise P_{ST} values at single morphometric trait D4_6 **(A)** and trait D10_9 **(B)** over female samples.

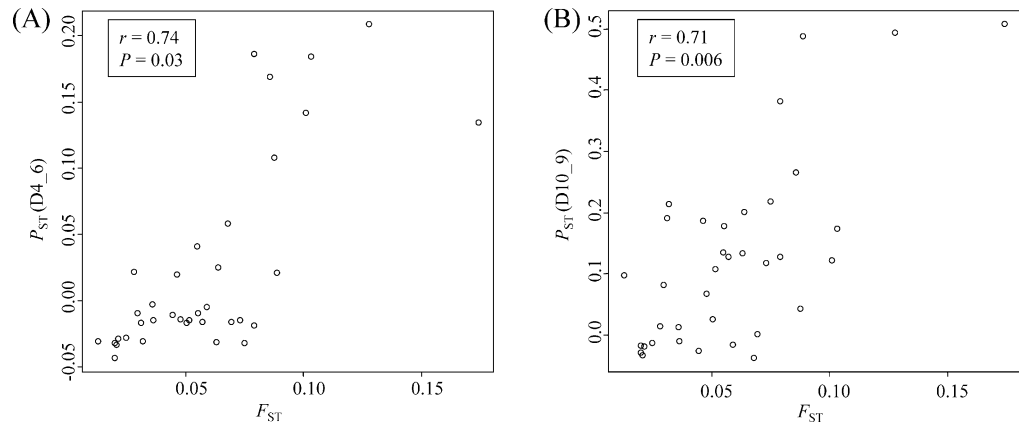## DISCUSSION

Sexual dimorphism

The present study revealed sexual dimorphism in *G. rarus* as approximately 64% of all the morphometric traits had significant sexual differences. From the distribution of these morphometric variables, it was found that the distances related to landmark 3 (pelvic-fin insertion) of *G. rarus* such as BD (body depth), D2_3, D3_1, D4_3, D3_6 and D5_3 were the most important discriminant factors for sexes. The sexual differences of these morphometric traits were in accordance with the findings in Wang (1992). Wang (1992) found that females usually have a plumper abdomen

and a relatively larger body size than males, which is a normal phenomenon in fish (Roff, 1983; Henderson et al., 2003; Kitano et al., 2007). Moreover, Wang (1992) revealed that the distance between the end of pectoral fin rays and the start point of the ventral fin in females of adult *G. rarus* is approximately one to four scales longer than in males (distance of one to two scales); the distance between the end of ventral fin rays and the cloacal aperture in females is approximately the distance of one to three scales longer than in males (approximately half a scale or sometimes the end of ventral fin rays could reach the cloacal aperture). On the whole, this sexual dimorphism highlights the main differences in the depth of the body shape of *G. rarus*.

Sexual dimorphism in external morphology includes differences in size, coloration, or body structure, which could result from different reproductive roles, niche divergence

between the sexes, preferences of one sex for particular traits of the other sex, and intra-sexual competition (Andersson, 1994; Kitano et al., 2007; Aguirre et al., 2008). The relatively larger size of females than males is common in *G. rarus*, which may be explained by several hypotheses including natural selection for greater egg production and sexual selection based on male preference for larger females. Studies have also revealed that sex-determinant mechanisms of *G. rarus* are complicated and may be influenced by environmental factors (You et al., 2010). Therefore, further research on mechanisms responsible for sexual dimorphism of *G. rarus* should be conducted in the future.

Population differentiation

Although populations were significantly differentiated in females and males according to truss network morphometric traits, we found a certain degree of trait overlap between almost all population pairs. It implied that the degree of population differentiation was not large enough to fully account for their isolation. He et al. (2012) also revealed that a weak but significant geographic isolation between those populations existed within the past few generations, but these may be due to the same historical source. However, it is worth mentioning that population T2 in males (especially) and in females seems to show the highest level of isolation, which could be confirmed by high accuracy of its prediction in males (100%).

From the distribution of most contribution traits in the body shape of *G. rarus*, it can be found that almost all the truss network traits played important roles in discriminating wild populations. Shao et al. (2007) also revealed that morphological differences between wild populations and inbred strains of *G. rarus* were obviously greater than those among wild populations. These morphological differentiations mainly reflected environmental differences between natural and laboratory conditions. Morphological variation in fish is largely induced by environmental factors, such as rearing temperature, water velocity, food quantity, food type or feeding mode, but not genetic factors (Beacham, 1990; Holopainen et al., 1997; Páez et al., 2008). Through long-term investigation on the habitats of *G. rarus*, the morphological differences between its wild populations are unlikely due to regime or diet, but rather likely due to water velocity.

Our results demonstrate that population differentiation of *G. rarus* involves not only morphological traits but also neutral genetic markers. He et al. (2012) revealed low to moderate levels of genetic differentiation and a weak but significant isolation-by-distance pattern among these nine wild populations of *G. rarus*, which were highly correlated with the evolution of rivers.

Comparisons between neutral genetic differentiation (F_{ST}) and phenotypic variation (phenotypic differentiation in the wild populations should be measured as P_{ST}) are increasingly being used in many population studies (Merilä and Crnokrak, 2001; Leinonen et al., 2006; Johansson et al., 2007; Chapuis et al., 2008; Jensen et al., 2008). There are usually three possible interpretations for the comparison: if $P_{ST} > F_{ST}$, directional natural selection must be involved to achieve the differentiation; if $P_{ST} = F_{ST}$, the observed quantitative differentiation could be obtained by genetic drift alone; if $P_{ST} < F_{ST}$, the observed degree of differentiation is

actually expected on the basis of selection favoring the same phenotype in different populations (Merilä and Crnokrak, 2001). The majority of studies comparing P_{ST} and F_{ST} typically identify diversifying selection as a driver of phenotypic divergence in specific traits of interest (Storz, 2002; Leinonen et al., 2008; Young et al., 2011). In fact, these possible explanations of this comparison rest on many assumptions; for example, it is usually assumed that phenotypic variance equals additive genetic variance, excluding nonadditive genetic and environmental effects (viz., $c = h^2$; Merilä, 1997; Brommer, 2011), otherwise they are problematic (Pujol et al., 2008). The critical premise is that local environments differ enough to allow selection acting on additive genetic variation to drive phenotypic divergence of populations. It is likely that different populations experience different environmental conditions, which will create inequality of c and h^2 (Pujol et al., 2008). The ratio c/h^2 is the critical aspect that describes how well P_{ST} approximates Q_{ST}, determining the robustness of the conclusions from the comparison of P_{ST} with the neutral expectation (Brommer, 2011). Therefore, interpretation of the P_{ST}-F_{ST} comparison results in the wild populations should be done with caution.

The present study revealed significant correlations between P_{ST} over two single traits (D4_6 and D10_9) in female samples and F_{ST}, which could mean that the differentiation of these two traits seems to follow the evolution of a neutral trait only affected by genetic drift. However, there were no significant correlations between P_{ST} over most traits and F_{ST}, hinting that most of the observed phenotypic discreteness may be not due to genetic drift. Besides, P_{ST} values clearly exceeded F_{ST} values among wild populations of *G. rarus*. Furthermore, the habitats of *G. rarus* are usually unstable, which is mainly due to environmental changes in the wild (He and Wang, 2010). That is, environmental influences could be the main drivers of phenotypic divergence in *G. rarus*. In general, many studies have proposed that fish usually demonstrate greater variance in morphological traits and are more susceptible to environmental factors than other vertebrates (Allendorf, 1988; Thompson, 1991; Turan et al., 2006; Langerhans et al., 2007; Young et al., 2011). Environmental influences on traits can arise via two evolutionary processes including natural selection (genetically based responses to selection) as well as phenotypic plasticity (potentially plastic responses to the environment) (Pigliucci, 2001; DeWitt and Scheiner, 2004; Langerhans et al., 2007). Phenotypic plasticity, usually understood as environmentally-induced phenotypic variation, is a major means of adaptation to environmental heterogeneity (Páez et al., 2008; Gianoli and Valladares, 2012). After a change in environment, plasticity can produce a partially adaptive phenotype, which subsequently becomes further adapted by natural selection (Baldwin, 1896; Young et al., 2011). Although our study does not allow the distinction between phenotypic plasticity and natural selection, it is likely that one of these two evolutionary processes (or both) contribute to the observed morphological patterns in *G. rarus*. More work including experimental studies (i.e., rearing experiments or assessment of local adaptation of coding genes underlying trait variation) would be needed to decipher the relative importance of these processes.

In conclusion, *G. rarus* shows patterns of morphological

variation between sexes and among populations. Phenotypic plasticity and natural selection are two evolutionary processes that are likely to contribute to such morphological divergence, the relative importance of which should be investigated with continued research. Knowledge of the patterns of morphological divergence and its driving mechanisms are crucial to the understanding of important evolutionary phenomena and ecological dynamics.

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