Genomic divergence, introduction history and latitudinal adaptation of grass carp

Le Wang *,†,§, Yubang Shen *,†,§, Jianjun Fu‡, Xiaoyan Xu*, Gen Hua Yue †,††,‡‡ and Jiale Li *

* Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai 201306, China

† Molecular Population Genetics Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore, 117604, Republic of Singapore

‡ Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081

†† Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Republic of Singapore

‡‡ School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Republic of Singapore

§ Le Wang and Yubang Shen contributed equally to this study

NGS data was deposited in the DDBJ Sequence Read Archive (Project accession no. PRJDB4785).

Keywords: grass carp, genomic divergence, introduction, local selection, latitudinal variation

Running title: population genomics of grass carp

Corresponding authors

Dr. Gen Hua Yue

Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604 Singapore

Tel: +65-68727405; Fax: +65-68727007; Email: genhua@tll.org.sg

Dr. Jiale Li

Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai 201306, China

Tel: +86 021 61900566; FAX: +86 021 61900405; E-mail: jlli2009@126.com

Abstract

Understanding the genomic signatures of population differentiation is fundamental to obtain a comprehensive view of the evolutionary process of organisms. Grass carp is one of the most important fish species in the world due to its significant value in aquaculture and world-wide vegetation biocontrol. However, little is known about the contemporary population structure and also the genetic basis of adaptation to a wide range of latitudinal environments. Using 43310 SNPs generated by genotyping by sequencing in 197 grass carps from nine populations, we examined the patterns of population differentiation, historical introduction and evidence of local selection. The overall genetic differentiation across all native populations was unexpectedly low. Nevertheless, these native populations were clearly differentiated into three genetic clusters, corresponding to the Yangtze River, the Pearl River and the Heilongjiang River System, respectively. Populations in Malaysia, India and Nepal, with the earliest introduction records, most likely have an origin from the Pearl River System. Using conceptually different approaches, 451 loci were detected under potential local selection, among which 84 were annotated to have a gene feature. 19.0% of the genes under putative selection were involved in immune responses, while 42.9% of the annotated loci showed a signature of latitudinal variation. This study provides valuable information for application of genomic tools in addressing questions concerning population differentiation that was influenced by both neutral and adaptive forces, as well as human activities.

Introduction

Grass carp (*Ctenopharyngodon idella*), belonging to the family Cyprinidae, is a large herbivorous freshwater fish species (Froese and Pauly 2015). It is of great importance as a food fish species as well as a species for world-wide aquatic vegetation control (Cross 1969; Lembi *et al.* 1978). Grass carp is native to eastern Asia and broadly distributed from the

Heilongjiang River System (Amur River) southward to northern Vietnam (Froese and Pauly 2015). According to literature records, grass carp has a culture history of more than 1300 years since the Tang Dynasty (FAO 2014). The aquaculture practices are mainly conducted within the geographical regions of the Yangtze and the Pearl River Systems of China (FAO 2014). Recent success in artificial breeding has significantly promoted the aquaculture industry of this species (Stanley 1976; Boney *et al.* 1984; Allen Jr and Wattendorf 1987; Peter *et al.* 1988). The annual global production has been over 5 million tons since 2013 with an estimated economic value of 5 billion US dollar (FAO 2014). Grass carp is of the highest production yield among all the farmed fish species around the world and accounts for approximately 15.6% of global freshwater aquaculture production (FAO 2014).

Due to herbivorous habits, grass carp has been broadly introduced to more than 40 countries around the world to control the undesirable and/or invasive aquatic plants of freshwater systems (Skelton 2001). Artificial introductions were intensively conducted since the 1960s (Welcomme 1988). The earliest introduction of grass carp was documented from Southern China to Malaysia by Chinese immigrants in the 1800s (Welcomme 1988). However, which native populations these introduced grass carp originated from is unclear. Recently, many studies have reported that introduced/invasive grass carp have endangered native ecological systems and caused great economic loss because they can completely eliminate vegetation from freshwater systems, destroy the populations of native fish species and introduce parasites (Moyle 1986; Chilton II and Muoneke 1992; Bain 1993).

Within its native distribution range, grass carp mainly lives in three independent river systems: the Heilongjiang River, the Yangtze River and the Pearl River System (Fu *et al.* 2013). Understanding range-wide population structure is critical to conserve and utilize the genetic resources (Avise 1992). However, it is still not clear, due to a lack of genetic studies (Zhang *et al.* 2001; Liu *et al.* 2009; Fu *et al.* 2013). In particular, grass carp has been cultured

for more than 1300 years (FAO 2014). It is also not known if aquaculture practices with such long history have left significant imprints on the contemporary population structure. Importantly, a geographical pattern of population differentiation is the genetic basis to trace the introduction from native habitats to foreign environments (Cornuet *et al.* 1999; Paetkau *et al.* 2004). Investigation on the environments of both native and foreign habitats can provide critical biological information for setting up effective introduction plans (Lande 1988; Bain 1993). It can also mitigate the adverse effects on the foreign habitats that were caused by grass carp as an agent of biological invasion (Cross 1969; Bain 1993).

Local selection is of critical importance in the evolution of species (Savolainen *et al.* 2013). Genetic studies focusing on different environments can provide crucial information for understanding the selective forces driving local adaptation (Sultan and Spencer 2002). In some cases, however, local selection acts along certain geographical gradients, e.g. latitude, longitude and altitude, and shows the same direction as the overall neutral forces (Storz 2002; Vasemägi 2006). Thus, it is more challenging to discriminate adaptive evolutionary forces from background neutral forces (McKay and Latta 2002; Storz 2002). Nevertheless, these environmental factors are of great interest in studying the selective forces that shape adaptive divergence (Gilchrist and Partridge 1999; Alberto *et al.* 2013). Significant associations between environmental variables and genetic markers are typically investigated and considered as footprints of local selection (Sezgin *et al.* 2004; Vasemägi 2006; Antoniazza *et al.* 2010). Although adaptive variation can be consistent with “isolation-by-distance” in geographical pattern and also explained by neutral forces like gene flow, genetic drift and admixture between adjacent populations, the footprints of local selection still can be inferred by comparing the relative pattern and strength of population differentiation against environmental variables between candidate loci of adaptive divergence and neutral markers (Storz 2002; McKown *et al.* 2014).

Grass carp are naturally distributed in a wide range from Southern Siberia to northern Vietnam spanning approximately 30 latitudinal degrees and is tolerant of extreme temperature from 0° to 38°C (Froese and Pauly 2015). The distribution is much likely limited by habitat temperature, and even correlated to the latitudinal variation of temperature. Due to the small number of markers, e.g. microsatellites, however, previous studies only focused on identifying the neutral genetic variations and population structure (Zhang *et al.* 2001; Liu *et al.* 2009; Fu *et al.* 2013). The lack of useful high-density genetic markers limited our understanding on adaptive population differentiation, particularly along latitude (Narum *et al.* 2013).

Here, a total of 197 grass carp, including both wild populations throughout the whole distribution range and populations with the earliest introduction history were analyzed using ddRAD-Seq approach (Peterson *et al.* 2012). First, the aim was to identify range-wide population structure and examine the pattern of gene flow, which can help understand the geographic and demographic factors that have played crucial roles in population differentiation of grass carp. Furthermore, we intended to test if the level of population differentiation is sufficient to trace the introduction of grass carp by using the populations with the earliest introduction history. The results can provide useful information for tracing global introduction of grass carp and better utilizing this species in the biocontrol of aquatic vegetation. In addition, as the native populations show a significant latitudinal distribution pattern, our goal was to detect the footprints of local selection and discuss if such divergence was correlated to specific environmental factors, particularly temperature. Finally, we aimed to identify candidate genes under potential directional selection, which help understand the mechanism of evolution under selection. In total, this study can disentangle the effects of demographic history, gene flow and local selection on the contemporary population differentiation and provide important information for both utilizing this species as a tool in

biocontrol and understanding the adaptive divergence of freshwater fish species in the presence of complicated gene flow and demographic history.

Materials and Methods

Sampling and data collection

Grass carp including six wild and three introduced populations consisting of 197 individuals were collected between 2007 and 2008. The wild populations were from the three river systems: the Heilongjiang River, the Yangtze River and the Pearl River System, across this species' distribution range, while the three introduced populations were sampled from Malaysia, India and Nepal, respectively (**Table 1 & Figure 1**). The annual average temperature of each sampling site was retrieved from weather.sina.com.cn (**Table 1**). Population Malaysia was documented as being introduced in the1800s from southern China, while population India was recorded as being introduced from Hong Kong, China (the Pearl River System), in 1959 and 1968 (Welcomme 1988). Population Nepal was set up by introduction from India in 1966-1967 (Shireman and Smith 1983). All samples were estimated as more than one year old. Fin tissue was collected and preserved in 95% ethanol at -20°C. Genomic DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen, Germany) and quantified using Qubit® assays (Life Technologies, USA).

Genotyping by sequencing

Genotyping by sequencing (GBS) was conducted using the ddRAD-Seq approach (Peterson *et al.* 2012). Restriction enzymes PstI-HF and MspI (New England Biolabs, USA) were selected for library construction. 200 ng genomic DNA was fully digested with two enzymes. Digested fragments were ligated with barcoded adaptors using T4 ligase (New England Biolabs, USA) and then pooled for cleanup with QIAquick PCR Purification Kit (Qiagen,

Germany). The cleaned products were size selected and purified (300-500 bp) by running gels and using QIAquick Gel Extraction Kit (Qiagen, Germany), respectively. The recovered libraries were then amplified using Phusion® High-Fidelity DNA Polymerase (New England Biolabs, USA). After a final cleanup using QIAquick PCR Purification Kit (Qiagen, Germany), the libraries were sent to a NextSeq 500 platform (Illumina, USA) for 2x150 bp paired-end sequencing.

The program *process_radtags* (Catchen *et al.* 2011) was employed to filter the raw sequencing reads with default parameters and reads with any uncalled base were removed. Clean reads were then demultiplexed and trimmed to 100 bp for *in silico* mapping. First, reads were mapped to the reference genome of grass carp v1.0 (Wang *et al.* 2015b) using the program BWA-MEM with default parameters (Li and Durbin 2010). Reads with multiple targets in the reference were excluded from further analysis. Reference-aligned reads were then assembled into stacks for each individual using *pstacks* implemented in the package Stacks v1.34 (Catchen *et al.* 2011). A total of 54 individuals randomly selected from each population were used to construct a catalogue of stacks using *cstacks*. Stacks from each individual were then matched against the catalogue for SNP discovery using *sstacks*. Finally, genotyping was conducted across all populations using the program *populations* with a minimum of 10× sequence depth. SNPs were further filtered to meet the following criteria: present in > 70% of the individuals in each population, have no more than two alleles and show an observed heterozygosity of < 0.5 (Hohenlohe *et al.* 2010). Only one SNP was retained for each RAD locus. Hardy-Weinberg equilibrium (HWE) for each locus was examined using Genepop v4.2 (Raymond and Rousset 1995) and loci that deviated from HWE in a single population at the significance level of 0.01 were excluded from further analysis.

Population structure and phylogenetic relationship

Genetic diversity for each population was measured by observed heterozygosity (H_O), expected heterozygosity (H_E) and nucleotide diversity (Π), while genetic divergence for each individual locus was estimated using *F-statistics* (Weir and Cockerham 1984). All these calculations were performed using the program *populations* (Catchen *et al.* 2011). Population genetic divergence was estimated in the form of pairwise F_{ST} using the program Arlequin 3.5 (Excoffier and Lischer 2010). Statistical significance was examined using an exact test with 10 000 permutations. Population structure at both the population and individual levels was investigated by principle component analysis using the program Eigenstrat v5.1 (Price *et al.* 2006). The pattern of population differentiation was examined in the form of isolation-by-distance (IBD) using Mantel tests with the program IBD v1.52 (Bohonak 2002). The genetic distance was measured using $F_{ST}/1\text{-}F_{ST}$, while the geographical distance was estimated as the linear distance between sampling localities.

The phylogenetic relationship among populations was constructed using a Neighbor-Joining approach with the program Populations v1.2.33 (Langella 1999) by bootstrapping over loci for 1000 times. The origins of introduced populations from native populations were determined using ancestral alleles. The software fastStructure v1.0 (Raj *et al.* 2014) was employed to infer the ancestral alleles between one introduced population and two native populations. The two selected native populations were from the Yangtze River and the Pearl River systems, respectively, and had the closest phylogenetic relationship with the introduced population. The program was run 10 times for each K value (from 1 to 6) with default parameters. The most likely number of genetic clusters (K) was estimated by plotting the marginal likelihood value.

Identifying footprints of selection

In order to identify evidence of latitudinal variation at SNP loci, we independently estimated the association between allele frequencies for each SNP and latitude at the population level using a liner correlation method. Pairwise genetic distance based on allele frequencies of individual locus was estimated according to the method by Reynolds *et al.* (1983). Genetic distance was then correlated to geographical distance among populations using Mantel tests to discriminate neutral mutations from the loci showing latitudinal variation in allele frequencies. By removing the loci under putative neutral processes, a set of candidate SNPs showing latitudinal variation but not isolation-by-distance in genetic divergence was obtained.

Evidence of local adaptation was detected for individual locus using a Bayesian generalized linear mixed model involving covariance of allele frequencies and environmental variables with the program Bayenv (Coop *et al.* 2010). A Bayes factor (BF) was calculated for each SNP to measure the strength of the correlation between SNP variation and environmental variables. According to the method by Coop *et al.* (2010), a BF > 3 was considered as a substantial evidence for selection. The program was run for five times with an independent variance-covariance matrix of population genetic variation to achieve consistency among the runs.

F_{ST}-based outlier tests were also performed to identify signatures of spatial purifying selection. Outlier loci under directional selection are expected to show higher levels of divergence, while loci under balancing selection would show lower levels of genetic divergence compared to the putative neutral loci (Beaumont and Nichols 1996; Foll and Gaggiotti 2008). Firstly, a Bayesian simulation-based test implemented in BayeScan (Foll and Gaggiotti 2008) was used to identify outlier SNPs. Loci with Bayes factor > 3 were considered as substantial outliers. Considering that grass carp are distributed in different river systems and that there is among-group genetic structure, a hierarchical island model (Beaumont and Nichols 1996) for identifying outlier loci was also employed using the

program Arlequin 3.5 (Excoffier and Lischer 2010) with the following parameters: 50 000 simulations, 10 simulated groups, and 100 demes per group. Only outliers above the 99% quantile of the null distribution were considered as candidates under spatial purifying selection.

Analysis of the genes under putative selection

Loci under putative directional selection were functionally annotated by Blast2Go (Conesa *et al.* 2005) against all available nucleotide databases with an E-value cutoff of 10^{-6}. SNPs within both exons and introns were considered to have a gene feature. Enrichment of Gene Ontology (GO) terms was conducted using the program WEGO (Ye *et al.* 2006) with default parameters. Loci were also mapped to the reference genome of zebrafish (GRCz10) using Blastx to retrieve the corresponding Ensembl gene IDs. A more detailed functional annotation of these genes were then performed by mapping to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto 2000) using the program David (Huang *et al.* 2009). The signaling pathways of at least two genes in default were enriched for further analysis.

Results

SNP discovery and genotyping

In total, NGS produced an average of 10.17 million raw reads for each individual. After quality control, 9.21 million reads per individual were obtained for sequence mapping and SNP discovery. A total of 280544 SNPs were discovered across all nine populations. After removing the loci that failed to meet the filtering criteria, 43310 SNPs were genotyped across all populations, among which 35844 (82.8%) showed minor allele frequency (MAF) of > 0.05.

Genetic diversity and population structure

The measures of genetic diversity, including H_O, H_E and Π, estimated based on all variant SNPs were shown in **Table 1**. For the six wild populations, Vietnam showed slightly lower genetic diversity than the others. We also observed that the genetic diversity of the introduced populations including Malaysia, India and Nepal were significantly lower than that of the wild populations ($P < 0.001$, one-way ANOVA test).

Pairwise F_{ST} analysis revealed significant genetic differentiation between the wild populations and the introduced populations with F_{ST} ranging from 0.1126 between Zhaoqing and India to 0.2399 between Vietnam and Malaysia (**Table 2**). However, genetic differentiation between each pair of wild populations was shallow with F_{ST} ranging from 0.0073 between Jiujiang and Shishou to 0.0515 between Hanjiang and Vietnam, although significantly different from 0. The wild population Zhaoqing from the Pearl River System showed slightly lower genetic differentiation with the three introduced populations: Malaysia, India and Nepal, compared to the other wild populations ($P < 0.05$, paired *t-test*). For wild populations, genetic divergence at individual locus was estimated between the most divergent populations (Hanjiang vs Vietnam) and also between the most distant populations (Nenjiang vs Vietnam). We observed that no loci showed $F_{ST} > 0.5$ and only < 15 % of loci had $F_{ST} > 0.1$ for both population pairs (**Figure 2**). Loci showing $F_{ST} > 0.1$ were found more frequently between Nenjiang and Vietnam (14.5%) than between Hanjiang and Vietnam (5.6%). Principle component analysis revealed that the wild populations were strikingly differentiated from the introduced populations (**Figure 3a**). For the wild populations, Zhaoqing and Vietnam from the Pearl River System were clearly differentiated from the populations from both the Yangtze River System (Hanjiang, Jiujiang and Shishou) and the Heilongjiang River System (Nenjiang), although there was some mixture of individuals between the Yangtze River and the Heilongjiang River Systems (**Figure 3b**). The pattern of genetic differentiation

284 across all wild populations rejected the model of isolation-by-distance (R^2 = 0.187, P = 0.16;
285 **Figure 4a**). Considering that some individuals of the Heilongjiang River System very likely
286 have an origin from the Yangtze River System (**Figure 3b**), the population Nenjiang was
287 further removed from Mantel tests. Interestingly, we identified a strong correlation between
288 population differentiation and geographical distance (R^2 = 0.876, P < 0.001; **Figure 4b**).

289 **Population introduction history**

290 The phylogenetic tree showed that native populations from the Yangtze River (Hanjiang,
291 Jiujiang and Shishou), the Heilongjiang River (Nenjiang) and the Pearl River (Zhaoqing and
292 Vietnam) Systems formed three independent clusters, respectively, with the Heilongjiang
293 River System cluster located between the Yangtze River System and the Pearl River System
294 clusters (**Figure 5a**). For the introduced populations, India and Nepal formed one subcluster
295 and joined into the Pearl River System cluster. Although joined into the Pearl River System,
296 the introduced population Malaysia showed a relatively long genetic distance with the other
297 populations within this cluster and was relatively close to the Heilongjiang River System.
298 Considering the results of the principle component analysis, population Nenjiang and
299 Malaysia might have an origin of admixture between the Yangtze River and the Pearl River
300 System lineages (**Figure 5a**). We further inferred the origin of the two populations separately
301 using ancestral alleles with the program fastStructure. Two populations of the closest genetic
302 relationship, Jiujiang and Zhaoqing from the Yangtze River and the Pearl River System,
303 respectively, were selected as the potential ancestral populations. Considering most of the
304 SNPs have a very low level of genetic divergence (**Figure 2**), only loci of F_{ST} > 0.05,
305 numbering 5986, were used for these analyses. The most likely K values for estimation of the
306 origins of the populations Nenjiang and Malaysia were inferred as 2 and 3, respectively
307 (**online supporting Figure S1**). We found that many more ancestral alleles in the Nenjiang
308 population in the Heilongjiang River System originated from the Yangtze River System than

from the Pearl River System (**Figure 5b**). However, for the introduced population Malaysia, only ancestral alleles from the Pearl River System (Zhaoqing) were observed (**Figure 5c**).

Identifying loci under putative selection

Among all the SNPs with MAF > 0.05, 5197 (14.4%) were found to have significant linear regression (P<0.05) between latitudinal gradients and allele frequencies. Mantel tests revealed that 3351 (9.4%) SNPs showed significant patterns of isolation-by-distance (R^2 > 0.514, P < 0.05) for individual locus. As stated above, the Nenjiang population showed a signature of admixture, and this produced decisive effects on the pattern of isolation-by-distance (**Figure 3 & Figure 4**). Therefore, we further removed this population from Mantel tests. Using five native populations, 3710 (10.4%) SNPs were observed to have significant patterns of isolation-by-distance (R^2 > 0.632, P < 0.05). After removing loci with any evidence of isolation-by-distance, a total of 2700 loci that showed significant latitudinal variation in allele frequencies were obtained for further analysis (**Figure 6**).

The Bayesian generalized linear mixed model identified 768 SNPs that showed significant association between genetic variations and latitudinal gradients across all six populations at individual locus (BF > 3). In F_{ST} based outlier tests, BayeScan detected 263 SNPs as substantial outliers (BF > 3), while the hierarchical island model identified 744 SNPs as significant outliers at the significance level of 0.99. A total of 791 (2.2%) unique loci were revealed to be outliers by the two F_{ST} based tests.

Within the 2700 loci that showed correlation to latitudinal gradients, 132 were candidates under directional selection as revealed by the program Bayenv and both F_{ST} based outlier tests (**Figure 6**). Moreover, Bayenv and the outlier tests identified 265 and 285 unique candidates under spatially purifying selection, respectively (**Figure 6**). We observed that the loci under putative selection revealed by the two outlier tests and Bayenv had much higher

genetic divergence with mean F_{ST} values of 0.198 and 0.104, respectively. However, the loci correlated to latitudinal gradients showed a much lower mean F_{ST} (0.034) than the loci with the pattern of IBD (F_{ST}, 0.065) and also the whole dataset (F_{ST}, 0.037) (**Figure 7**). Low genetic divergence much likely suggests that selection pressure is weak on these loci. In order to reduce false positives, only loci that were revealed to be under potentially directional selection by outlier tests, Bayenv or allele frequencies association study, and also showed F_{ST} of more than the 95% quantile (0.121) of the whole dataset, were retained, which produced 451 loci for further analyses.

Functional annotation of genes under putative selection

Among the 451 loci, 84 (18.6%) were annotated as having a gene feature and were further investigated (**Table S1**). 42.9% (36) of the annotated genes were indicated to be significantly associated with latitudinal gradients as revealed by Bayenv or allele frequency correlation study. GO enrichment revealed that these genes covered a wide range of functions in biological processes: biological regulation, cellular process, developmental process, immune system process, metabolic process, pigmentation and response to stimulus (**online supporting Figure S2**). Three KEGG pathways: Focal adhesion, Vascular smooth muscle contraction and the Toll-like receptor signaling pathway, were enriched with each containing two genes (**Table S2**). Interestingly, by searching for literature, we found 16 (19.0%) genes that play important roles in immune responses (e.g. MHC I UAK and MHC II DAB) and/or antiviral responses (e.g. Myxovirus resistance proteins and Mitochondrial antiviral signalling protein) (**Table 3**). Among these immune-related genes, 8 (50.0%) showed a pattern of latitudinal variation as revealed by Bayenv or allele frequency correlation study, while the other 8 genes were suggested to be under spatially purifying selection (**Table 3**).

Discussion

In this study, we investigated range-wide population structure of native populations and origin of introduced populations in the South and Southeast Asia, as well as latitudinal variation and local selection of grass carp using population genomic approaches. This study provides important implications for future application of grass carp in the biocontrol of aquatic vegetation and understanding the mechanism of local adaptation, particularly adaptive latitudinal variation for freshwater fish species with a wide range of geographical distribution.

High gene flow among native populations

Typically, freshwater fishes are more isolated by various geographical factors than marine fishes and thus have a lower level of gene flow (Ward *et al.* 1994). In this study, we observed that pairwise population genetic differentiation was very low (F_{ST}, 0.0073-0.0515), comparable to a previous study using microsatellites (Liu *et al.* 2009). This level of gene flow across the native populations of grass carp is much higher than the average for freshwater species (Gyllensten 1985; Ward *et al.* 1994; Cooke *et al.* 2012) and even higher than some fishes living in the open marine environment, e.g. the Chinook salmon (*Oncorhynchus tshawytscha*) (Larson *et al.* 2014), Atlantic salmon (*Salmo salar*) (Bourret *et al.* 2013) and Asian seabass (*Lates calcarifer*) (Wang *et al.* 2015a). Further investigation on genetic differentiation at individual locus revealed that only < 15 % of total loci showed $F_{ST} > 0.1$ and no loci had $F_{ST} > 0.5$. Considering the geographical isolation among the three river systems, such results are rather unexpected. The genetic distance between the Pearl River System (Vietnam) and the Yangtze River System (Hanjiang) was closer than between the Pearl River System (Vietnam) and the Heilongjiang River System (Nenjiang), although the latter showed a rather longer geographical distance. Nevertheless, we observed that the number of loci with $F_{ST} > 0.1$ was much more between Nenjiang and Vietnam than between Hanjiang and Vietnam. Considering the long aquaculture history of more than 1300 years,

these contradicting results strongly suggest that the high gene flow among the three river systems is not only naturally occurring but also induced by human activities. Interestingly, the observed pattern of population differentiation did not conform to isolation-by-distance across the whole data set. However, after removal of the Nenjiang population that was suggested to have an admixture origin between the Yangtze River and the Pearl River Systems, the remaining populations showed a strong signal of isolation-by-distance. This indicates that although human-induced gene flow might have played important roles in shaping the overall population structure of grass carp, it only showed overwhelming importance in the Heilongjiang River System.

According to historical records, grass carp was abundant in both the Yangtze River System and the Pearl River System, and was widely captured from the wild as seeds for aquaculture locally (FAO 2014). There was no practical need to introduce grass carp between the two river systems. On the other hand, it is reasonable that gene flow can be high between the two river systems because they partially overlap in geography (Zhu 1993). For these reasons, the migration occurred much more naturally and thus the population differentiation showed a strong pattern of isolation-by-distance. However, we cannot exclude the possibility that human activities played important roles in dispersal of grass carp. Such gene flow might be merely induced so randomly with no directional purpose that it has much less effects on shaping genetic structure than the natural gene flow.

In contrast to the Yangtze and the Pearl River systems, the distribution and culture of grass carp in the Heilongjiang River System have never been abundant nor considered as a major aquaculture practice according to both historical records and current official fishery statistics (FAO 2014; Liu and Li 2015). Geographically, the Heilongjiang River System is completely isolated with the other two river systems. On the other hand, the Nenjiang population was mixed with alleles originating from both the Yangtze River and the Pearl River Systems as

407 inferred from fastStructure. Hence, the low genetic differentiation between this river system
408 and the other river systems strongly indicates that human-induced dispersal played more
409 important roles than natural introgression. In fact, grass carp in the Heilongjiang River
410 System grow slower than in the other river systems due to low water temperature (Cui *et al.*
411 1995). Therefore, seeds from the other river systems, particularly from the Yangtze River
412 system, were commonly introduced for aquaculture purposes because of geographical
413 adjacency. This is the most likely explanation for the low genetic differentiation of grass carp
414 between the Heilongjiang River System and the other river systems.

415 **Origins of the early introduced populations**

416 Both in terms of genetic diversity and differentiation, we observed significant genetic
417 heterogeneity between all of the native populations and the introduced populations including
418 Malaysia, India and Nepal, suggesting significant founder effects in the introduction of these
419 populations (Barton and Charlesworth 1984). It is likely that the introduction of grass carp
420 was not initiated under planned programs or that not all the introduced fish can adapt to the
421 new habitats. Genetic differentiation among the native populations is the basis to trace the
422 origins of the introduced populations (Cornuet *et al.* 1999; Paetkau *et al.* 2004). As expected,
423 we identified significant genetic differentiation and also a clear geographical pattern of
424 population differentiation among the three river systems in a background of high gene flow.
425 Native populations from the Heilongjiang River, the Yangtze River and the Pear River
426 Systems were separately clustered into independent genetic lineages, although there was
427 evidence of population admixture for the Heilongjiang River System. These results provided
428 critical clues to trace back population origins. Both pairwise F_{ST} and phylogenetic analyses
429 indicated that all three introduced populations, Malaysia, India and Nepal originated from the
430 Pearl River System, which was also supported by the data inferred from ancestral alleles.

First, the Pearl River System is geographically more adjacent to Malaysia, India and Nepal than the Yangtze River and the Heilongjiang River Systems. Therefore, it is reasonable that the Pearl River System was preferred as the source for introduction to these countries. In contrast, the Yangtze River and the Heilongjiang River Systems are not only distant from Southeast and the South Asia, but also isolated by various continental barriers, e.g. the Himalaya Mountains. It is a great challenge to introduce fish from these two river systems to Southeast and the South Asia. Most importantly, it was recorded that grass carp was first introduced into Malaysia from Southern China with the large-scale migration of Chinese people in the 1800s, although it is not clear which river system the Malaysia population originated from (Welcomme 1988). The introduction history could be inferred from the routes of Chinese migration during that time. As revealed by history studies, most of the Chinese people in Southeast Asia were from Guangdong and Fujian provinces (Pan 1999), which geographically overlap with the Pearl River System. Thus, the Malaysia population very likely has an origin in the Pearl River System, consistent with the results of the genetic data.

In total, our data suggest that the native populations might have accumulated enough genetic divergence for population origin assignment of the recently introduced populations of grass carp, e.g. the populations introduced to Europe, North America and also some Southern Hemisphere countries (Mitchell 1986). These results are very valuable for studying the production and physiological adaptation, as well as the living environments and habitat preferences, of both native and introduced populations. Such information can be referenced to construct comprehensive introduction plans in the future.

Local selection and latitudinal variation

454 It is a great challenge to discriminate local selection from neutral processes for organisms that
455 have experienced complicated demographic history. Neutral processes can generate the same
456 marks on genomic architecture as local selection does (Storz 2002; Vasemägi 2006;
457 Savolainen *et al.* 2011; Wang *et al.* 2013; McKown *et al.* 2014; Hornoy *et al.* 2015). In some
458 cases, adaptive traits show a specific distribution pattern along specific environmental factors.
459 If the estimates of neutral forces are coincidentally varying along the same environmental
460 factors, the difficulty of disentangling the roles of adaptive driving forces would be greatly
461 enhanced (Merilä and Crnokrak 2001; McKay and Latta 2002; Storz 2002). Under this
462 condition, a single association test between an individual locus and an environmental factor is
463 obviously not enough to determine if one locus has experienced spatially divergent selection,
464 particularly in the background of genome-wide patterns of isolation-by-distance (Vasemägi
465 2006). Grass carp is such a species, which has a significant signature of latitudinal
466 distribution. Thus, the adaptive traits might vary in parallel with the pattern of neutral
467 processes along specific geographical gradients, like latitude. These evolutionary processes
468 limited the potential to identify the molecular mechanism underlying adaptive evolution
469 (McKay and Latta 2002; Chen *et al.* 2012). Here, we used conceptually different approaches
470 to differentiate the footprints of local selection from the currents of neutral evolutionary
471 processes (Hansen *et al.* 2010; Wang *et al.* 2013).

472 Our main purpose was to identify individual loci of higher genetic divergence than can be
473 explained by random genetic drift and gene flow (Storz 2002). As discussed above, grass carp
474 from different river systems very likely have unique demographic history. Grass carp
475 originated from the Yangtze River System and expanded into the Pearl River and the
476 Heilongjiang River Systems during the Pleistocene and Pliocene, respectively (Li and Fang
477 1990). Nevertheless, the contemporary population structure was significantly shaped by high
478 levels of gene flow due to both natural and artificial factors. As the Pearl River System and

479 the Heilongjiang River System cover the southernmost and the northernmost distribution
480 ranges, respectively, such contrasting environments have likely posed strong selective
481 pressure on the distribution of grass carp (Gardner and Latta 2006). Gene flow within the
482 Pearl River and the Yangtze River Systems might be seldom influenced by human activities,
483 as population differentiation still shows a significant pattern of isolation-by-distance.
484 However, gene flow between the Heilongjiang River System and the other two river systems
485 were profoundly influenced by recent human activities, which overall changed the
486 geographical pattern of population differentiation such that the pattern of isolation-by-
487 distance was no longer observed. Although influenced by human activities, the extreme
488 northernmost environmental condition of the Heilongjiang River System can pose strong
489 selective pressure on the introduced grass carp. Such a process of natural selection provides
490 important clues to discriminate footprints of natural selection from genome-wide patterns of
491 isolation-by-distance.

492 Consistent with the overall neutral evolutionary process, a large number of loci, 6489 (18.1%)
493 of the total loci, were indicated to show a pattern of isolation-by-distance in genetic
494 divergence. Although 14.4% (5197) were revealed to have significant correlations between
495 latitudinal gradients and allele frequencies, some of them would be false positives because
496 these loci also showed significant correlations between pairwise geographical distance and
497 genetic divergence. In total, these results suggest a strong background of isolation-by-
498 distance in the overall population differentiation of grass carp. After removing the loci which
499 were potential false positives by application of a series of conceptually different approaches,
500 only 451 loci were suggested to be under putative directional selection, accounting for 1.3%
501 of the total loci. This ratio is much less than in previous studies (2.3%-10%) using fish
502 species that showed weak or non-significant genome-wide patterns of isolation-by-distance,
503 like Atlantic salmon (Bourret *et al.* 2013), Chinook salmon (Larson *et al.* 2014) and

yellowfin tuna (*Thunnus albacares*) (Grewe *et al*. 2015). Such results likely suggest that it is much less efficient to identify loci under putative directional selection with a genome-wide pattern of isolation-by-distance (Beaumont and Nichols 1996). Among the loci under putative directional selection, 18.6% were revealed to be associated with functional genes. Interestingly, 42.9% (36) of the annotated genes were indicated to be significantly correlated to latitudinal gradients, indicating clinally adaptive divergence at these loci (Storz 2002; Vasemägi 2006; Chen *et al.* 2012). Although these genes are involved in various functions, we observed a significant cluster of genes (16, 19.0%) playing important roles in immune responses (e.g. MHC I UAK and MHC II DAB) (Benacerraf 1981) and/or antiviral responses (e.g. Myxovirus resistance proteins and Mitochondrial antiviral signalling protein) (Seth *et al.* 2005; Gao *et al.* 2011). Among these immune-related genes, 8 (50.0%) showed a pattern of latitudinal adaptive variation. This result further suggests that the distribution of grass carp spanning approximately 30 latitudinal degrees was also the consequence of clinal adaptation along latitude. In the case of grass carp, the annual average temperature was observed to be highly correlated to the latitude of the sampling sites (R^2 = 0.992, P < 0.001). However, we did not find any evidence that the enriched genes were associated to thermal adaptation. This result might suggest that the latitudinal adaptive distribution of grass carp was not directly selected by the temperature. Because the enriched genes were observed to have functions in defense against various pathogens and the diversity of pathogens were strongly related to the environmental temperature (Cashdan 2001; Mitchell *et al.* 2005; Dionne *et al.* 2007), our results might suggest that the latitudinal adaptation or clinal adaption of grass carp along latitude was the consequence of selection by pathogens and indirectly by temperature of the habitats.

In total, the joint application of different approaches identified a promising set of loci that were under putative directional selection. Many of them have a pattern of latitudinal

529 variations. The latitudinal distribution of grass carp likely has an adaptive genetic basis,
530 although the underlying causes remain to be elucidated. Nevertheless, spatially purifying
531 selection has played important roles in shaping the contemporary population structure of
532 grass carp. Our data shed light on the genetic basis of local adaptation of grass carp with a
533 large distribution range.

534 **Acknowledgements**

535 We thank Mr. Narayan Prasad Pandit and Mr. Dang Hai Nguyen for sample collection from
536 Vietnam, Indian and Nepal respectively. This research is supported by the National Key
537 Technology R&D Program of China (2012BAD26B02) and the China Agriculture Research
538 System (CARS-46-04) foundations.

539 **Authors' contributions**

540 YS, LW, JL and GHY conceived the study and finalized the manuscript. YS and LW
541 designed the experiments. YS, JF and XX carried out the lab experiments. LW and YS
542 performed bioinformatics, analysed the molecular data and drafted the manuscript. All
543 authors have read and approved the final manuscript.

544 **References**

545 Alberto, F. J., J. Derory, C. Boury, J.-M. Frigerio, N. E. Zimmermann *et al.*, 2013 Imprints of
546 natural selection along environmental gradients in phenology-related genes of
547 *Quercus petraea*. Genetics 195**:** 495-512.
548 Allen Jr, S. K., and R. J. Wattendorf, 1987 Triploid grass carp: status and management
549 implications. Fisheries 12**:** 20-24.

Antoniazza, S., R. Burri, L. Fumagalli, J. Goudet and A. Roulin, 2010 Local adaptation maintains clinal variation in melanin-based coloration of European barn owls (*Tyto alba*). Evolution 64**:** 1944-1954.

Avise, J. C., 1992 Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63**:** 62-76.

Bain, M. B., 1993 Assessing impacts of introduced aquatic species: grass carp in large systems. Environmental Management 17**:** 211-224.

Barton, N. H., and B. Charlesworth, 1984 Genetic revolutions, founder effects, and speciation. Annual Review of Ecology and Systematics 15**:** 133-164.

Beaumont, M. A., and R. A. Nichols, 1996 Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London B: Biological Sciences 263**:** 1619-1626.

Benacerraf, B., 1981 Role of MHC gene products in immune regulation. Science 212**:** 1229-1238.

Bohonak, A., 2002 IBD (isolation by distance): a program for analyses of isolation by distance. Journal of Heredity 93**:** 153-154.

Boney, S. E., W. L. Shelton, S.-L. Yang and L. Wilken, 1984 Sex reversal and breeding of grass carp. Transactions of the American Fisheries Society 113**:** 348-353.

Bourret, V., M. P. Kent, C. R. Primmer, A. Vasemägi, S. Karlsson *et al.*, 2013 SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). Molecular Ecology 22**:** 532-551.

Cashdan, E., 2001 Ethnic diversity and its environmental determinants: Effects of climate, pathogens, and habitat diversity. American Anthropologist 103**:** 968-991.

574 Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko and J. H. Postlethwait, 2011 Stacks:
575 building and genotyping loci de novo from short-read sequences. G3: Genes,
576 Genomes, Genetics 1**:** 171-182.
577 Chen, J., T. Källman, X. Ma, N. Gyllenstrand, G. Zaina *et al.*, 2012 Disentangling the roles of
578 history and local selection in shaping clinal variation of allele frequencies and gene
579 expression in Norway spruce (*Picea abies*). Genetics 191**:** 865-881.
580 Chilton II, E. W., and M. I. Muoneke, 1992 Biology and management of grass carp
581 (*Ctenopharyngodon idella*, Cyprinidae) for vegetation control: a North American
582 perspective. Reviews in Fish Biology and Fisheries 2**:** 283-320.
583 Conesa, A., S. Götz, J. M. García-Gómez, J. Terol, M. Talón *et al.*, 2005 Blast2GO: a
584 universal tool for annotation, visualization and analysis in functional genomics
585 research. Bioinformatics 21**:** 3674-3676.
586 Cooke, G. M., N. L. Chao and L. B. Beheregaray, 2012 Divergent natural selection with gene
587 flow along major environmental gradients in Amazonia: insights from genome scans,
588 population genetics and phylogeography of the characin fish *Triportheus albus*.
589 Molecular Ecology 21**:** 2410-2427.
590 Coop, G., D. Witonsky, A. Di Rienzo and J. K. Pritchard, 2010 Using environmental
591 correlations to identify loci underlying local adaptation. Genetics 185**:** 1411-1423.
592 Cornuet, J.-M., S. Piry, G. Luikart, A. Estoup and M. Solignac, 1999 New methods
593 employing multilocus genotypes to select or exclude populations as origins of
594 individuals. Genetics 153**:** 1989-2000.
595 Cross, D., 1969 Aquatic weed control using grass carp. Journal of Fish Biology 1**:** 27-30.
596 Cui, Y., S. Chen and S. Wang, 1995 Effect of temperature on the energy budget of the grass
597 carp, *Ctenopharyngodon idellus*. Oceanologia et Limnologia Sinica 26**:** 169-174.

598 Dionne, M., K. M. Miller, J. J. Dodson, F. Caron and L. Bernatchez, 2007 Clinal variation in
599 MHC diversity with temperature: evidence for the role of host–pathogen interaction
600 on local adaptation in Atlantic salmon. Evolution 61**:** 2154-2164.
601 Excoffier, L., and H. E. Lischer, 2010 Arlequin suite ver 3.5: a new series of programs to
602 perform population genetics analyses under Linux and Windows. Molecular Ecology
603 Resources 10**:** 564-567.
604 FAO, 2014 Cultured Aquatic Species Information Programme *Ctenopharyngodon idellus*,
605 Rome, www.fao.org/fishery/culturedspecies/Ctenopharyngodon_idellus, (accessed
606 10/2015).
607 Foll, M., and O. Gaggiotti, 2008 A genome-scan method to identify selected loci appropriate
608 for both dominant and codominant markers: a Bayesian perspective. Genetics 180**:**
609 977-993.
610 Froese, R., and D. Pauly, 2015 FishBase. World Wide Web electronic publication,
611 www.fishbase.org, (accessed 10/2015).
612 Fu, J., J. Li, Y. Shen, R. Wang, Y. Xuan *et al.*, 2013 Genetic variation analysis of wild
613 populations of grass carp (*Ctenopharyngodon idella*) using microsatellite markers.
614 Hereditas 35**:** 192-201.
615 Gao, S., A. von der Malsburg, A. Dick, K. Faelber, G. F. Schröder *et al.*, 2011 Structure of
616 myxovirus resistance protein a reveals intra-and intermolecular domain interactions
617 required for the antiviral function. Immunity 35**:** 514-525.
618 Gardner, K. M., and R. G. Latta, 2006 Identifying loci under selection across contrasting
619 environments in *Avena barbata* using quantitative trait locus mapping. Molecular
620 Ecology 15**:** 1321-1333.

621 Gilchrist, A. S., and L. Partridge, 1999 A comparison of the genetic basis of wing size
622 divergence in three parallel body size clines of *Drosophila melanogaster*. Genetics
623 153**:** 1775-1787.
624 Grewe, P., P. Feutry, P. Hill, R. Gunasekera, K. Schaefer *et al.*, 2015 Evidence of discrete
625 yellowfin tuna (*Thunnus albacares*) populations demands rethink of management for
626 this globally important resource. Scientific Reports 5**:** 16916.
627 Gyllensten, U., 1985 The genetic structure of fish: differences in the intraspecific distribution
628 of biochemical genetic variation between marine, anadromous, and freshwater species.
629 Journal of Fish Biology 26**:** 691-699.
630 Hansen, M. M., K. Meier and K. L. D. Mensberg, 2010 Identifying footprints of selection in
631 stocked brown trout populations: a spatio-temporal approach. Molecular Ecology 19**:**
632 1787-1800.
633 Hohenlohe, P. A., S. Bassham, P. D. Etter, N. Stiffler, E. A. Johnson *et al.*, 2010 Population
634 genomics of parallel adaptation in threespine stickleback using sequenced RAD tags.
635 PLoS Genetics 6**:** e1000862.
636 Hornoy, B., N. Pavy, S. Gérardi, J. Beaulieu and J. Bousquet, 2015 Genetic adaptation to
637 climate in white spruce involves small to moderate allele frequency shifts in
638 functionally diverse genes. Genome Biology and Evolution 7**:** 3269-3285.
639 Huang, D. W., B. T. Sherman and R. A. Lempicki, 2009 Systematic and integrative analysis
640 of large gene lists using DAVID bioinformatics resources. Nature Protocols 4**:** 44-57.
641 Kanehisa, M., and S. Goto, 2000 KEGG: kyoto encyclopedia of genes and genomes. Nucleic
642 Acids Research 28**:** 27-30.
643 Lande, R., 1988 Genetics and demography in biological conservation. Science 241**:** 1455-
644 1460.

Langella, O., 1999 POPULATIONS 1.2. 30 Population genetic software. CNRS UPR9034.[Online.] Available at bioinformatics. org/~ tryphon/populations.

Larson, W. A., L. W. Seeb, M. V. Everett, R. K. Waples, W. D. Templin *et al.*, 2014 Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). Evolutionary Applications 7**:** 355-369.

Lembi, C. A., B. G. Ritenour, E. M. Iverson and E. C. Forss, 1978 The effects of vegetation removal by grass carp on water chemistry and phytoplankton in Indiana ponds. Transactions of the American Fisheries Society 107**:** 161-171.

Li, H., and R. Durbin, 2010 Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26**:** 589-595.

Li, S., and F. Fang, 1990 Geographical distribution of the four kinds of pond-cultured carps. Acta Zoologica Sinica 36**:** 244-250.

Liu, F., J. H. Xia, Z. Y. Bai, J. J. Fu, J. L. Li *et al.*, 2009 High genetic diversity and substantial population differentiation in grass carp (*Ctenopharyngodon idella*) revealed by microsatellite analysis. Aquaculture 297**:** 51-56.

Liu, Z., and S. Li, 2015 *China Fisheries Yearbook 2014*. China Agriculture Press, Beijing, China.

McKay, J. K., and R. G. Latta, 2002 Adaptive population divergence: markers, QTL and traits. Trends in Ecology & Evolution 17**:** 285-291.

McKown, A. D., R. D. Guy, J. Klápště, A. Geraldes, M. Friedmann *et al.*, 2014 Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. New Phytologist 201**:** 1263-1276.

Merilä, J., and P. Crnokrak, 2001 Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14**:** 892-903.

Mitchell, C., 1986 Effects of introduced grass carp on populations of two species of small native fishes in a small lake. New Zealand Journal of Marine and Freshwater Research 20**:** 219-230.

Mitchell, S. E., E. S. Rogers, T. J. Little and A. F. Read, 2005 Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. Evolution 59**:** 70-80.

Moyle, P., 1986 Fish introductions into North America: patterns and ecological impact, pp. 27-43 in *Ecology of biological invasions of North America and Hawaii*. Springer, New York.

Narum, S. R., C. A. Buerkle, J. W. Davey, M. R. Miller and P. A. Hohenlohe, 2013 Genotyping-by-sequencing in ecological and conservation genomics. Molecular Ecology 22**:** 2841-2847.

Paetkau, D., R. Slade, M. Burden and A. Estoup, 2004 Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13**:** 55-65.

Pan, L., 1999 *The encyclopedia of the Chinese overseas*. Harvard University Press, USA.

Peter, R. E., H.-R. Lin and G. Van Der Kraak, 1988 Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture 74**:** 1-10.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher and H. E. Hoekstra, 2012 Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7**:** e37135.

Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick *et al.*, 2006 Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38**:** 904-909.

695 Raj, A., M. Stephens and J. K. Pritchard, 2014 fastSTRUCTURE: variational inference of
696 population structure in large SNP data sets. Genetics 197**:** 573-589.
697 Raymond, M., and F. Rousset, 1995 GENEPOP (version 1.2): population genetics software
698 for exact tests and ecumenicism. Journal of Heredity 86**:** 248-249.
699 Reynolds, J., B. S. Weir and C. C. Cockerham, 1983 Estimation of the coancestry coefficient:
700 basis for a short-term genetic distance. Genetics 105**:** 767-779.
701 Savolainen, O., S. T. Kujala, C. Sokol, T. Pyhäjärvi, K. Avia *et al.*, 2011 Adaptive potential
702 of northernmost tree populations to climate change, with emphasis on Scots pine
703 (*Pinus sylvestris* L.). Journal of Heredity 102**:** 526-536.
704 Savolainen, O., M. Lascoux and J. Merilä, 2013 Ecological genomics of local adaptation.
705 Nature Reviews Genetics 14**:** 807-820.
706 Seth, R. B., L. Sun, C. K. Ea and Z. J. Chen, 2005 Identification and characterization of
707 MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3.
708 Cell 122**:** 669-682.
709 Sezgin, E., D. D. Duvernell, L. M. Matzkin, Y. Duan, C.-T. Zhu *et al.*, 2004 Single-locus
710 latitudinal clines and their relationship to temperate adaptation in metabolic genes and
711 derived alleles in Drosophila melanogaster. Genetics 168**:** 923-931.
712 Shireman, J. V., and C. R. Smith, 1983 *Synopsis of biological data on the grass carp,*
713 *Ctenopharyngodon idella (Cuvier and Valenciennes, 1844)*. Food & Agriculture
714 Organization. Rome, Italy.
715 Skelton, P. H., 2001 *A complete guide to the freshwater fishes of southern Africa*. Southern
716 Book Publishers, Halfway House, South Africa.
717 Stanley, J. G., 1976 Production of hybrid, androgenetic, and gynogenetic grass carp and carp.
718 Transactions of the American Fisheries Society 105**:** 10-16.

719 Storz, J. F., 2002 Contrasting patterns of divergence in quantitative traits and neutral DNA
720 markers: analysis of clinal variation. Molecular Ecology 11**:** 2537-2551.
721 Sultan, S. E., and H. G. Spencer, 2002 Metapopulation structure favors plasticity over local
722 adaptation. The American Naturalist 160**:** 271-283.
723 Vasemägi, A., 2006 The adaptive hypothesis of clinal variation revisited: single-locus clines
724 as a result of spatially restricted gene flow. Genetics 173**:** 2411-2414.
725 Wang, L., S. Liu, Z. Zhuang, L. Guo, Z. Meng *et al.*, 2013 Population genetic studies
726 revealed local adaptation in a high gene-flow marine fish, the small yellow croaker
727 (*Larimichthys polyactis*). PLoS ONE 8**:** e83493.
728 Wang, L., Z. Y. Wan, H. S. Lim and G. H. Yue, 2015a Genetic heterogeneity and local
729 adaptation of Asian seabass across Indonesian Archipelago revealed with gene-
730 associated SNP markers. Fisheries Research 170**:** 205-211.
731 Wang, Y., Y. Lu, Y. Zhang, Z. Ning, Y. Li *et al.*, 2015b The draft genome of the grass carp
732 (*Ctenopharyngodon idellus*) provides insights into its evolution and vegetarian
733 adaptation. Nature Genetics 47**:** 625-631.
734 Ward, R., M. Woodwark and D. Skibinski, 1994 A comparison of genetic diversity levels in
735 marine, freshwater, and anadromous fishes. Journal of Fish Biology 44**:** 213-232.
736 Weir, B. S., and C. C. Cockerham, 1984 Estimating F-statistics for the analysis of population
737 structure. Evolution 38**:** 1358-1370.
738 Welcomme, R. L., 1988 *International introductions of inland aquatic species*. Food &
739 Agriculture Organisation of the United Nations. Rome, Italy.
740 Ye, J., L. Fang, H. Zheng, Y. Zhang, J. Chen *et al.*, 2006 WEGO: a web tool for plotting GO
741 annotations. Nucleic Acids Research 34**:** 293-297.

742 Zhang, S., H. Deng, D. Wang and L. Yu, 2001 Population structure and genetic diversity of
743 silver carp and grass carp from populations of Yangtze river system revealed by
744 RAPD. Acta Hydrobiologica Sinica 25**:** 330-336.
745 Zhu, D., 1993 *The Dictionary of River Systems in China*. Qingdao Press, Qingdao, China.

746

747

748

749

750

751

752

753

754

755

756

757

758

759

760

761 **Supporting information**

762 **Table S1** Summary statistics of annotated genes under putative directional selection and their
763 potential functions in grass carp

764 **Table S2** Enriched KEGG pathways and the candidate genes under potential selection in
765 grass carp

766

767

768 **Tables**

769 **Table 1** Sampling information of six native and three introduced grass carp populations
770 including river systems of origin, numbers of samples, sampling localities and dates, and the
771 annual average temperature of each sampling locality. Measures of genetic diversity
772 including observed heterozygosity (H_O), expected heterozygosity (H_E) and nucleotide
773 diversity (Π) are also indicated.

Samples	Origin	N	Longitude	Latitude	Date	Temperature	H_O	H_E	Π
Nenjiang	Heilongjiang River System	22	125.22	49.21	2007	4.3	0.197	0.202	0.207
Hanjiang	Yangtze River System	26	119.43	32.35	2007	15.4	0.200	0.201	0.205
Jiujiang	Yangtze River System	23	115.96	29.72	2007	17.7	0.202	0.205	0.210
Shishou	Yangtze River System	11	112.39	29.74	2007	16.9	0.210	0.204	0.214
Zhaoqing	Pearl River System	21	112.53	23.08	2007	22.7	0.203	0.201	0.206
Vietnam	Pearl River System	26	105.98	21.12	2008	24.8	0.190	0.191	0.195
Malaysia	Introduced	18	101.15	4.58	2008	Na.	0.136	0.126	0.130
India	Introduced	25	83.37	26.76	2008	Na.	0.171	0.168	0.171
Nepal	Introduced	25	85.03	27.42	2008	Na.	0.161	0.152	0.155

774

775

776 **Table 2** Pairwise F_{ST} values among each pair of populations of grass carp. Genetic
777 differentiation that was non-significant after Bonferroni corrections ($P < 0.001$) is denoted in
778 bold.

	Nenjiang	Hanjiang	Jiujiang	Shishou	Zhaoqing	Vietnam	India	Nepal	Malaysia
Nenjiang	-								
Hanjiang	0.0260	-							
Jiujiang	0.0228	0.0154	-						
Shishou	0.0196	0.0123	**0.0073**	-					
Zhaoqing	0.0272	0.0351	0.0337	0.0279	-				
Vietnam	0.0448	0.0515	0.0484	0.0449	0.0238	-			
India	0.1265	0.1307	0.1282	0.1289	0.1126	0.1297	-		
Nepal	0.1540	0.1580	0.1532	0.1596	0.1384	0.1592	0.1814	-	
Malaysia	0.2295	0.2255	0.2224	0.2336	0.2205	0.2399	0.3167	0.3501	-

779

780

781

782

783

784

785

786

787

788

789

790 **Table 3** Enriched candidate genes under putative directional selection and their potential
791 functions in grass carp. Approaches, including F_{ST} outlier tests, Bayenv and allele frequency
792 correlations (AFC), that were used to determine if one gene was under putative selection, are
793 also indicated.

Locus	Gene name	Function	Approaches		
Lca7796	Toll-like receptor 5b	Immune response	F_{ST} outlier		
Lca7927	Chemokine (C-C motif)-like	Immune response			AFC
Lca15228	DEXH (Asp-Glu-X-His) box polypeptide 58	Antiviral signaling	F_{ST} outlier		
Lca33879	Phosphofurin acidic cluster sorting protein 2	Antiviral signaling		Bayenv	
Lca52319	Myxovirus (influenza virus) resistance E	Antiviral signaling	F_{ST} outlier	Bayenv	AFC
Lca57825	Integrin alpha FG-GAP repeat containing 1	Antiviral signaling		Bayenv	AFC
Lca79226	Interleukin-1 receptor-associated kinase 1	Immune response	F_{ST} outlier		
Lca88973	Major histocompatibility complex class I antigen UKA	Immune response	F_{ST} outlier	Bayenv	AFC
Lca110035	Grass carp reovirus (GCRV)-induced gene 2l	Antiviral signaling	F_{ST} outlier		
Lca152085	Interferon induced with helicase C domain 1	Immune response	F_{ST} outlier		
Lca152284	PC4 and SFRS1 interacting protein 1	Antiviral signaling	F_{ST} outlier		AFC
Lca159757	Major histocompatibility complex class II DAB	Immune response	F_{ST} outlier		
Lca175136	Lymphocyte cytosolic protein 1	Immune response			AFC
Lca183026	Myxovirus (influenza) resistance A	Antiviral signaling	F_{ST} outlier		
Lca202544	Baculoviral IAP repeat containing 2	Immune response	F_{ST} outlier		
Lca285612	Mitochondrial antiviral signalling protein	Antiviral signaling			AFC

794

795

796

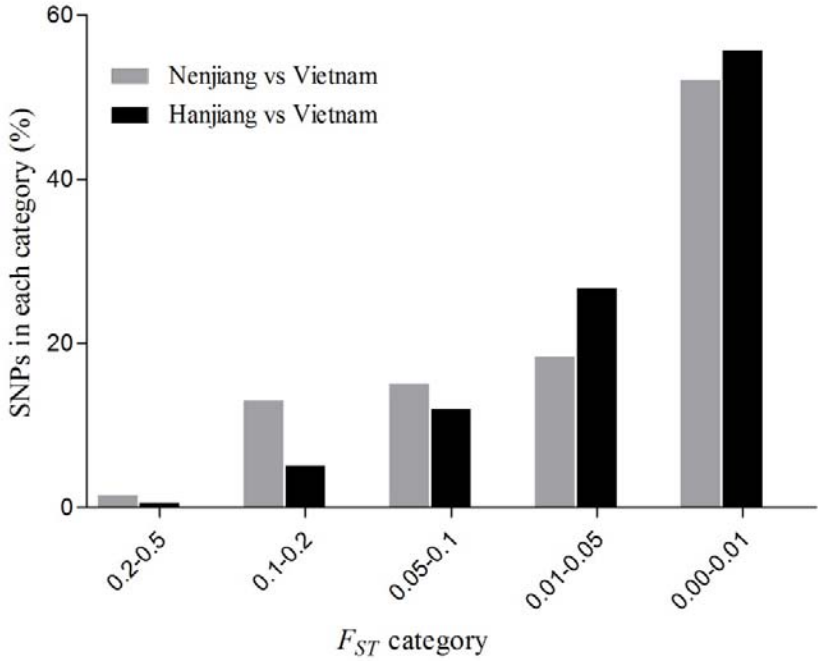
797

798

799

Figures

Figure 1 Sampling sites of six native grass carp populations distributed in the three river systems: the Heilongjiang River, the Yangtze River and the Pearl River Systems, and three introduced populations from Malaysia, India and Nepal. The wild and introduced populations are denoted as black and red solid circles, respectively. Detailed sampling information is listed in Table 1.



812

813 **Figure 2** Distribution of F_{ST} values in different categories between Nenjiang and Vietnam,
814 with the longest geographical distance, and between Hanjiang and Vietnam, with the largest
815 genetic distance, based on all genotyped SNPs with MAF > 0.05.

816

817

818

819

820

821

822

823

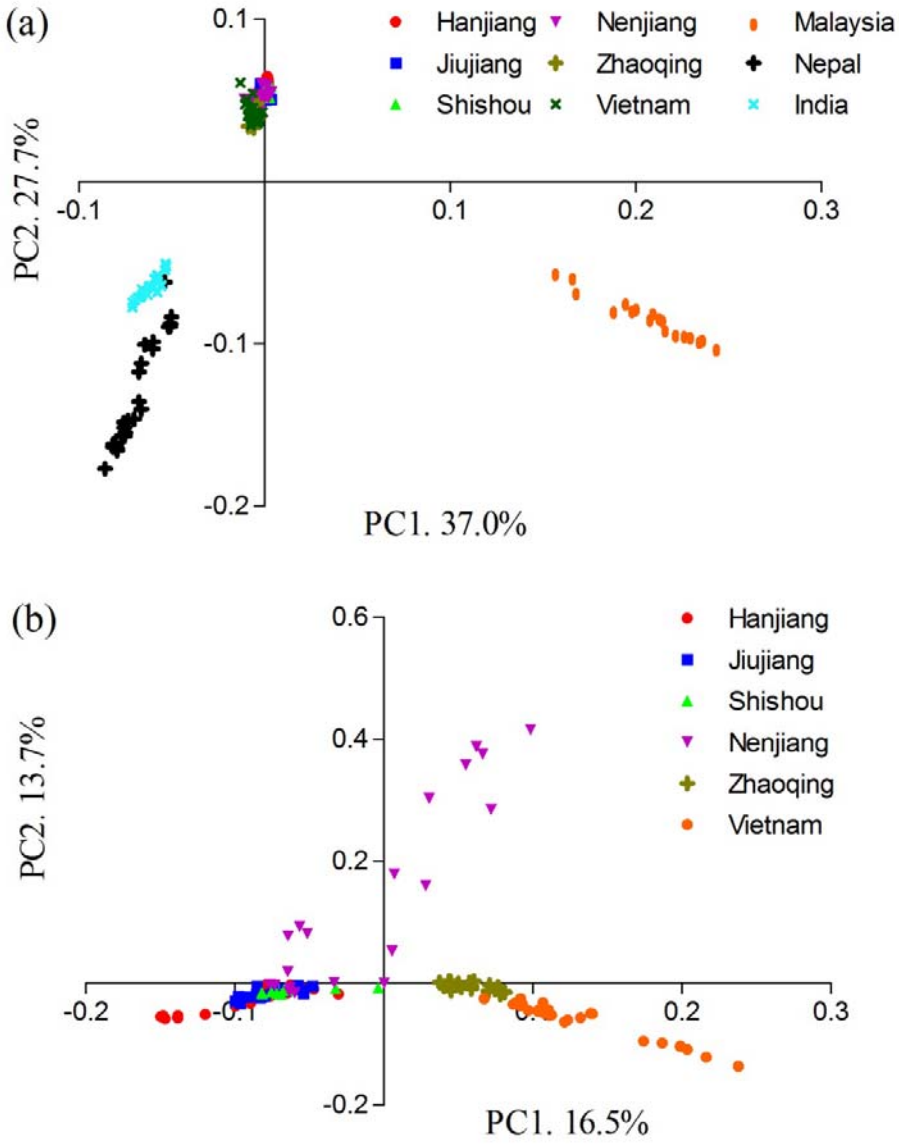


Figure 3 Principal component analyses for (a) all nine populations and (b) six native populations of grass carp based on all genotyped SNPs.

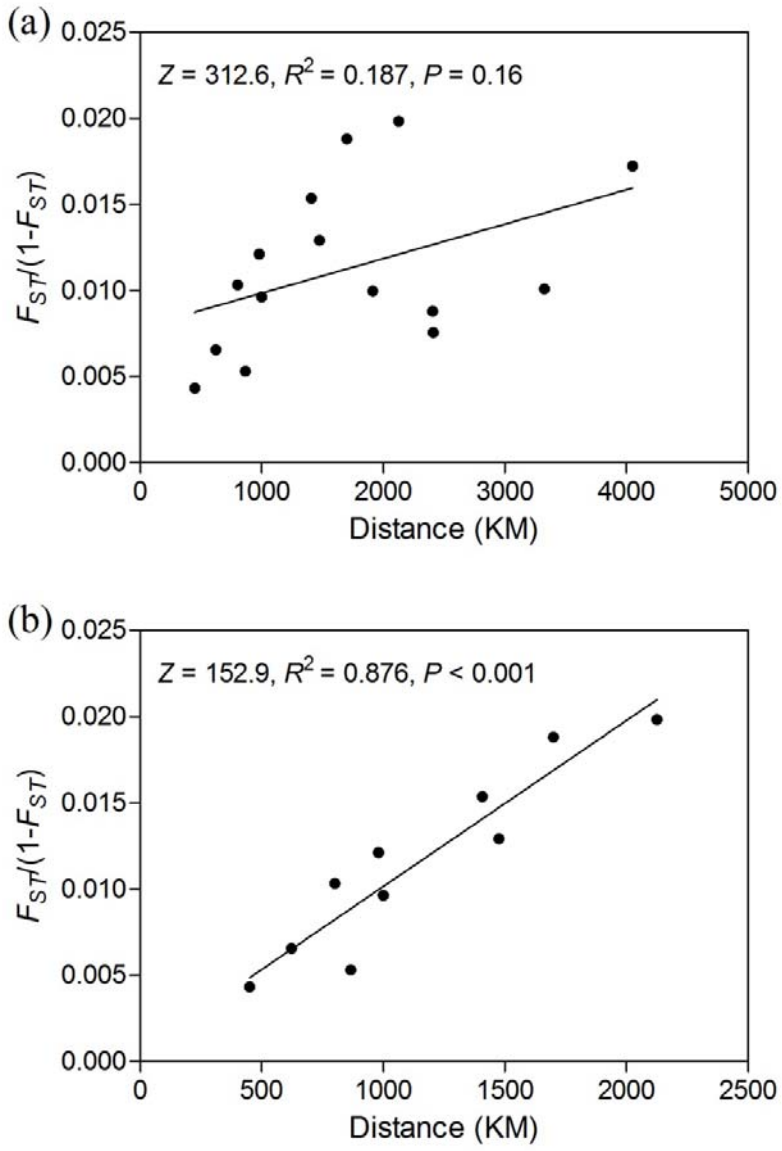
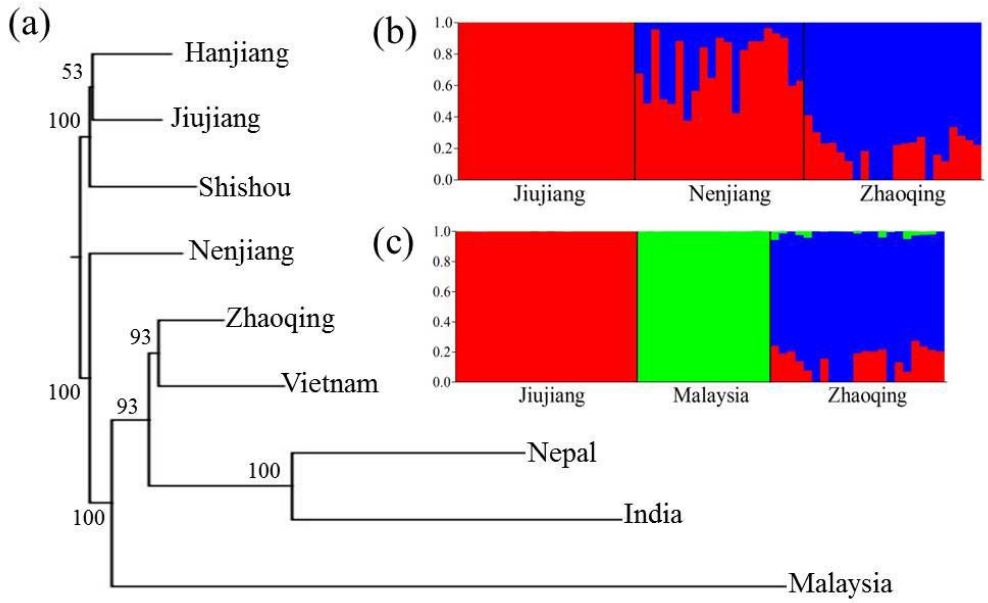


Figure 4 The overall pattern of isolation-by-distance for (a) all six native populations and (b) five native populations excluding population Nenjiang, examined using Mantel tests based on all genotyped SNPs. Genetic distance was estimated as $F_{ST}/(1-F_{ST})$, while geographical distance was the linear distance between sampling localities.

839



840

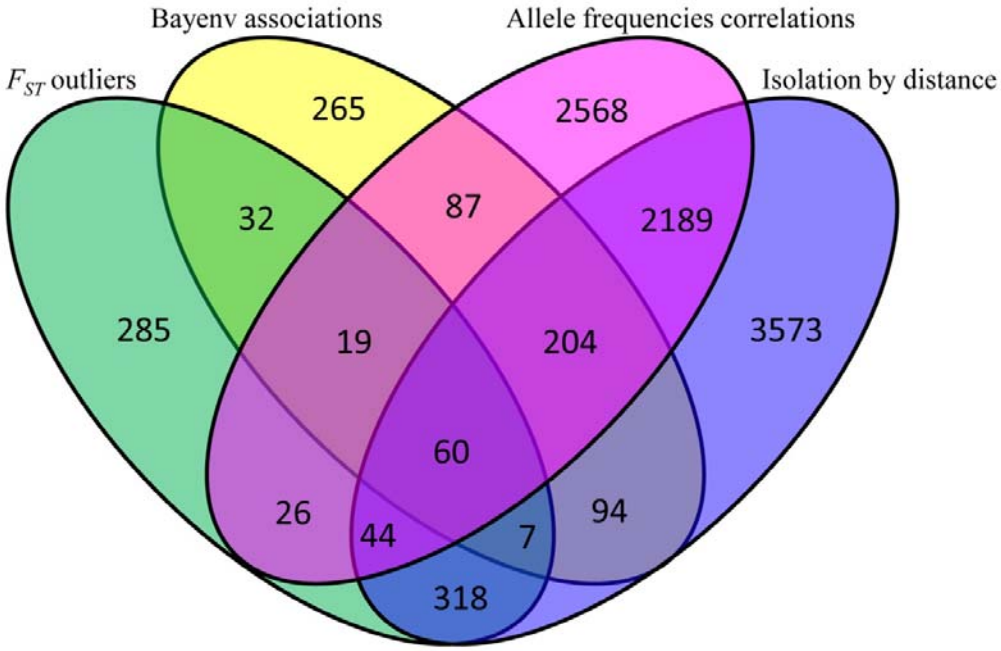
841 **Figure 5** (a) Phylogenetic relationships among all nine populations of grass carp constructed
842 using the Neighbor-Joining approach. Bootstrap supports over loci for 1000 times are
843 indicated. (b) Genetic assignment of the native population Nenjiang to the Yangtze River
844 System and the Pearl River System and (c) genetic assignment of the introduced population
845 Malaysia to the Yangtze River System and the Pearl River System, respectively. The most
846 likely K value for both assignment tests in the program Structure was inferred as 5. Each
847 vertical line represents one individual, while each colour shows the genetic composition that
848 is assigned into a distinct genetic cluster.

849

850

851

852



853

854 **Figure 6** Venn diagrams showing the number of loci under putative selection and isolation-
855 by-distance as revealed by F_{ST} outlier tests, Bayenv association tests, Allele frequency
856 correlations and Mantels tests for Isolation-by-distance. The numbers of overlapping loci
857 among the different approaches are also illustrated.

858

859

860

861

862

863

864

865

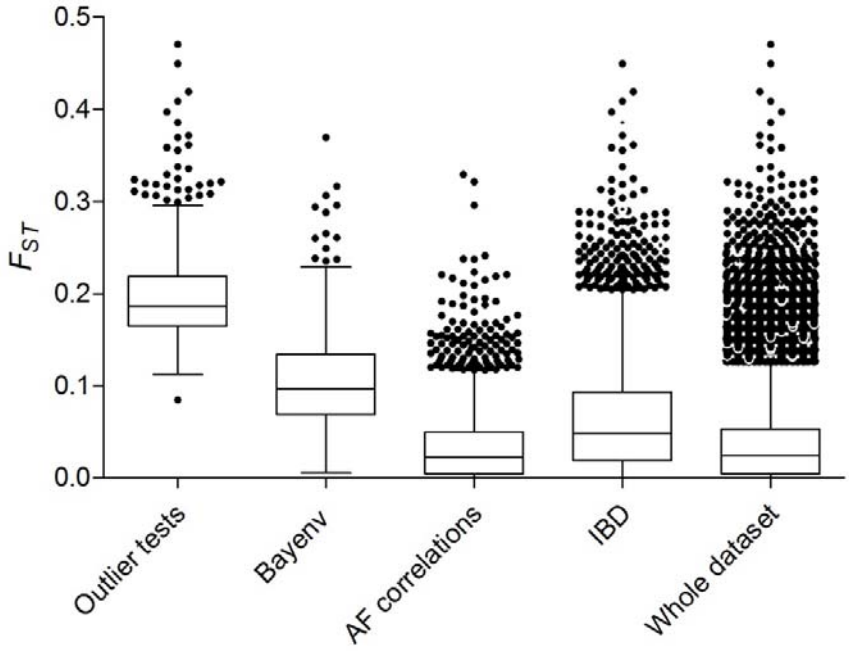


Figure 7 Distribution of F_{ST} values for the SNPs that were identified as outliers for putative directional selection, associated with latitude as revealed by Bayenv, had allele frequencies correlated to latitude and agreed with the pattern of isolation-by-distance, as well as for the whole dataset. The highest and lowest error bar indicates the 95% quantile, while the median horizontal line denotes the mean F_{ST} value. Individual locus with F_{ST} over the upper 95% quantile is shown.

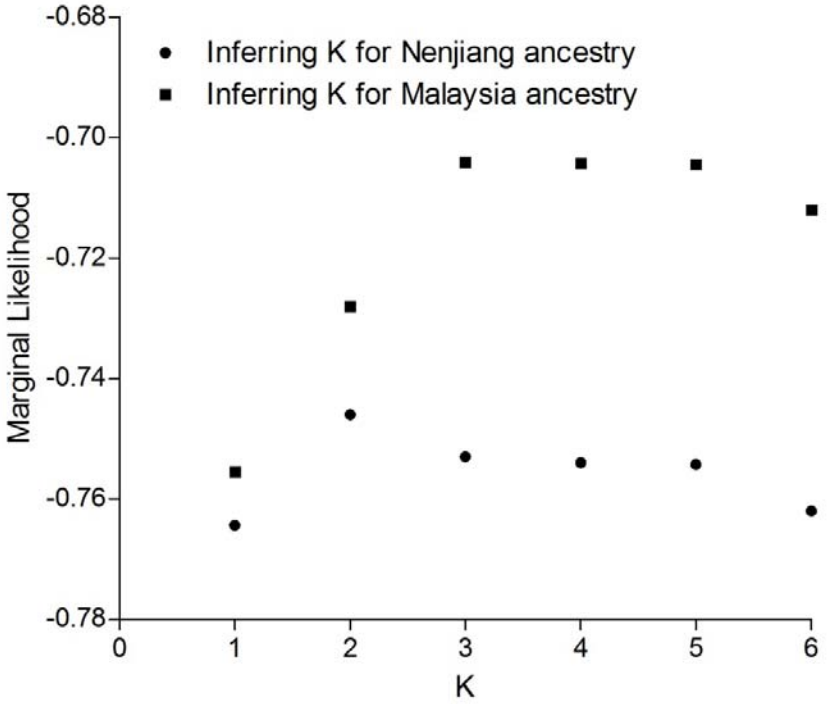
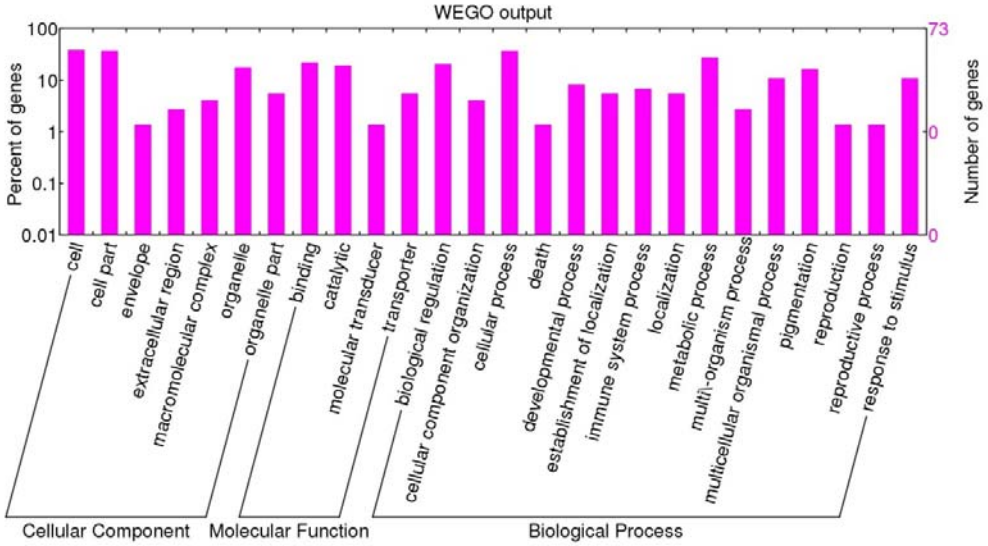


Figure S1 Plotting of K values inferred from the program fastStructure.



890

891 **Figure S2** Gene ontology annotations of the candidate genes under potential local selection

892 in grass carp. Three categories: Cellular Component, Molecular Function and Biological

893 Process, were used to visualize the potential functions of enriched genes

894