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Genetic differentiation of non-native populations of Gibel Carp, *Carassius gibelio* in Western Turkey by ISSR and SRAP markers

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Freshwater fish are one of the most frequently translocated and introduced aquatic animal groups and exhibit higher establishment ratios than many other taxa. Introductions are usually irreversible. One of common non-native fish species in Turkey is the Gibel Carp, *Carassius gibelio* which was introduced in the 1980s and is now widespread. We tested dominant markers (ISSR and SRAP) for genetic characterisation of Gibel Carp samples collected from eight locations in western Turkey. ISSR and SRAP marker sets showed that the level of gene flow between these populations (N_m = 0.45 / N_m = 0.47) is low and that the level of genetic differentiation (G_{ST} = 0.53 / G_{ST} = 0.52) is high. Inter-population variation detected by ISSR and SRAP markers constituted half part of the population (46.88 / 50.00%), while the rest was at intra-population level. These results indicate that the present population of the Gibel Carp is the result of several colonization events originating from the different sources. The phylogenetic relationship among the populations suggest that there were two independent major introduction events, one in the Marmara Region and the other in southern Turkey.

Keywords: Intra-population variation; non-native fish; molecular markers; genetic diversity

Introduction

Introduced species can be an ecological threat if they can successfully adapt into an ecosystem, resulting in potential negative interactions with local species and also impacting on ecosystem process (Gozlan & Newton, 2009). Fish species are one of the most frequently introduced aquatic organisms worldwide mainly because of social and economic demands for aquaculture, recreational fishing, ornamental purpose and commercial fisheries activities (Gozlan et al., 2010). Once introduced fish species become invasive, they can cause economic losses and have detrimental effects on habitats. Hence, they are considered a causal agent in the loss of biodiversity mainly by threatening native and endemic fishes (Townsend, 2003). Eradication and control of invasive species is generally troublesome and available management options are likely to threaten native species as well (Leprieur, Brosse, García-Berthou, Oberdorff, Olden, & Townsend, 2009).

Parallel to the worldwide status, Turkish inland waters are exposed to habitat degradation and species introductions that cause decreasing of endemic fish populations (Tarkan & Marr, 2015). Many introduced freshwater fishes have established widespread populations and threaten local and endemic species especially in fragile ecosystems.

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Gibel Carp *Carassius gibelio* (Bloch 1782), known as the most frequently introduced freshwater fish species in Turkey (e.g. Yerli et al., 2014; Tarkan & Marr, 2015; Ağdamar & Tarkan, 2019) have been reported to have negative impacts on native fauna on a large scale (Tarkan, Gaygusuz, Gürsoy Gaygusuz, Saç, & Copp, 2012). The main biological characteristics for invasiveness of Gibel Carp is its gynogenetic reproduction and tolerance to adverse environmental conditions such as low oxygen, turbidity and pollution (Tarkan et al., 2012). Previous studies have increasingly reported genetic variation observed in Gibel Carp populations in Turkey (e.g. Ağdamar & Tarkan, 2019) related to successful establishment and colonization of new habitats of the species (Harrison & Mondor, 2011). Since the first report of Gibel Carp in European part of Turkey some 40 years ago, the origin and distribution of the species has not fully been clarified, i.e. whether the present populations are descending from a single introduction or is the result of various introduction events. Therefore, it is not known if their populations are genetically homogenous.

To this end, we used dominant ISSR and SRAP molecular markers for differentiating Gibel Carp populations. Multi-locus marker systems are used to estimate genetic variation in different organisms and do not require a priori sequence information for molecular characterisation (Baysal et al., 2009; 2011). Introns, promotors and spacer sequences detected by ISSR primers (Baysal et al., 2011; Moghaieb et al., 2017) show high genetic variations within individuals belonging to the same species. A nucleic acid sequence that contains an above average number of Adenine and Thymine bases are also present in promotor and introns and the reverse primer 3' end of SRAP primers (Li & Quiros, 2001; Li, Gao, Yang, & Quiros, 2003; Robarts & Wolfe, 2014). Therefore, any polymorphism by these molecular markers is a basic, reliable, middle-yield and high-dominant total, iterative solution on detecting genetic variation of different species (Baysal et al., 2009; Devran & Baysal, 2012). These markers have also been used for tracking genetic diversity, identification, relationship and detection of invasion pathways and resources of alien species (Hebert, Cywinska, Ball, & DeWaard, 2003) and their use to discover variations in genetic structure of the fish species has been becoming common (Ji et al., 2014; Pechsiri & Vanichanon, 2015).

Material and Methods

Study areas and sampling. Gibel Carp samples (N=75) were collected from eight provinces in western Turkey between 2013 and 2014 (Figure 1, Table 1) including Thrace (European part of Turkey), where the species was firstly reported in Turkey in the 1980s (Tarkan et al., 2012). Specimens were collected by electrofihing (SAMUS 725P) and anaesthetized using 2-phenoxyethanol. Muscle tissue samples (dorsal part of the body) taken from each specimen were stored in 95% ethanol at -20°C until DNA extraction.

DNA extraction. Genomic DNA was extracted from muscğle tissue using Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA) according to protocol of the manufacturer. After the extraction, DNA samples checked in terms of quantity and purity with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) were visualized on 1% agarose gel in case of degradation possibility. Final concentrations were all set to 50 ng/µl to prevent PCR inhibition due to excess amount of template DNA. The acquired DNA samples were used either in PCR reaction or stored at -20°C.

DNA amplification. Three ISSR primers (ISSR1, ISSR3, and ISSR6) and six SRAP primer pair combinations (Me8-Em2, Me9-Em4, Me9-Em8, Me4-Em13, Me13-Em8, and Me8-Em15) were used for the amplification (Baysal et al., 2011; Devran & Baysal, 2012). PCR were conducted using Eppendorf Mastercycler®, in 50 µl volumes containing 5µl of 10X Taq Buffer with KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 5 µl of $MgCl_2$ (25 mM), 1µl of dNTPs (10 mM), 1µl

Table 1. List of provinces, sampling locations and population codes, number of specimens, and coordinates of Gibel Carp (*Carassius gibelio*) populations studied in western Turkey. N = Number of specimens.

Province	Sampling Location	Population Code	N	Latitude	Longitude
Antalya	Aksu River	AK	10	36°51'N	30°55'E
Aydın	Lake Azap	AL	5	37°35'N	27°26'E
Balıkesir	Lake Manyas	ML	10	40°14'N	27°55'E
Bursa	Lake Iznik	IL	10	40°24'N	29°42'E
Edirne	Meriç River	MR	10	41°10'N	26°31'E
İstanbul	Lake Büyükçekmece	BL	10	41°04'N	28°32'E
Muğla	Ula Reservoir	UR	10	37°07'N	28°23'E
Uşak	Üçpınarlar Reservoir	UL	10	38°25'N	28°58'E

of each primer (10 pM/µl), 2 U of Taq polymerase (5U/µl), and 4 µl of DNA (50 ng/µl). PCR amplifications were run in following thermal cycler conditions (Keskin & Atar, 2012): preliminary denaturation at 95°C for 5 min followed by 35 cycles consisting of denaturation at 95°C for 1 min, primer annealing temperatures between 54°C and 57°C for 1 min, primer extension at 72°C for 1 min and final extension step at 72°C for 10 min. PCR products were then run in a 1.5% agarose gel electrophoresis to examine the band corresponding to amplification product and negative control indicating possible contamination during the process.

Data analysis. All PCR yields were visualized by 1.5% high resolution agarose gel electrophoresis in 1xTBE buffer at 80 V for 120 minutes, stained ethidium bromide and photographed under UV light. Amplified bands from each primer were scored as present (1) or absent (0). The bands showing consistently amplifications were considered, smeared and weak bands were discarded from the analysis. Several statistics were used to evaluate Gibel Carp populations for intra- and interpopulation genetic diversity. Polymorphism, the percentage of polymorphic loci (*P*) detected (criterion of 99% was used), mean number of observed (n_a) and effective (n_e) alleles per locus (Kimura & Crow, 1964), Nei's gene diversity (*h*) as a measure of heterozygosity (Nei, 1973), and Shannon's information index (*I*) (Lewontin, 1972) were calculated. Total genetic variation (H_T), within-population genetic variation (H_S), and Nei's (1973) genetic differentiation coefficient (G_{ST}) were determined. Gene flow (N_m) was estimated from G_{ST} values using the relationship $Nm=0.5(1-G_{ST})/G_{ST}$, in which *N* is the effective population size and *m* is the proportion of the population that are migrants. Subsequently, genetic distances among all possible population pairs were calculated (Nei, 1972) and a dendrogram was constructed using the UPGMA (unweighted pair-group method with arithmetic average) method. All calculations were performed with POPGENE v1.32 (Yeh, Boyle, Rongcai, Ye, & Xiyan, 1999).

Results

Three ISSR primers generated a total of 38 well-resolved bands of which 97.37% were polymorphic in the 75 specimens sampled from 8 populations. The size of the amplified fragments ranged from 300 to 1,400 bp. Genetic parameters for intra- and interpopulation variability are given in Table 2. The percentage of polymorphic loci (*P*) varied in the populations between 23.68% (AL) and 63.16% (BL and UR – see Table 1 for abbreviations) with an overall mean of 44.74%. The overall mean number of observed alleles per locus (n_a) was 1.45±0.32, while the overall mean number of effective alleles per locus (n_e) was 1.26±0.33. AL had the lowest value for n_a (1.24±0.30), and BL and UR had the highest value (1.63±0.33). n_e values of the populations varied between 1.18±0.29 (MR and UL) and 1.38±0.35 (UR). The overall means of Nei's gene diversity (*h*) was 0.15±0.20 and Shannon's information index (*I*) 0.23±0.28. *h* values ranged from 0.11±0.17 in AL to 0.22±0.24 in UR, and *I* values ranged from 0.16±0.22 to 0.33±0.32 in the same populations. Total genetic variation (H_T) was 0.32±0.03. A

Table 2. Genetic parameters by molecular markers used (ISSR and SRAP) in Gibel Carp (*Carassius gibelio*) populations collected from western part of Turkish inlands. *P*: Percentage of polymorphic loci, n_a: Average of observed alleles, n_e: Average of effective alleles, *h*: Nei's gene diversity, *I*: Shannon information index. Average and standard deviation of all the considered parameters were calculated for ISSR and SRAP separately.

	P		***n_a***		***n_e***		***h***		***I***	
	ISSR	**SRAP**	**ISSR**	**SRAP**	**ISSR**	**SRAP**	**ISSR**	**SRAP**	**ISSR**	**SRAP**
AK	44.74	51.92	1.45±0.32	1.52±0.33	1.27±0.34	1.35±0.38	0.16±0.23	0.20±0.23	0.24±0.26	0.29±0.33
AL	23.68	17.31	1.24±0.30	1.17±0.25	1.22±0.32	1.13±0.33	0.11±0.17	0.07±0.11	0.16±0.22	0.10±0.18
ML	36.84	30.77	1.37±0.33	1.31±0.29	1.22±0.33	1.25±0.32	0.13±0.18	0.13±0.16	0.20±0.31	0.19±0.25
IL	55.26	30.77	1.55±0.36	1.31±0.30	1.27±0.35	1.17±0.31	0.16±0.21	0.10±0.15	0.27±0.33	0.11±0.20
MR	36.84	55.77	1.37±0.29	1.56±0.35	1.18±0.29	1.30±0.39	0.12±0.17	0.18±0.20	0.18±0.23	0.28±0.31
BL	63.16	38.46	1.63±0.33	1.39±0.32	1.34±0.35	1.24±0.33	0.21±0.25	0.14±0.19	0.32±0.35	0.21±0.25
UR	63.16	50.00	1.63±0.33	1.50±0.33	1.38±0.35	1.31±0.36	0.22±0.24	0.18±0.21	0.33±0.32	0.27±0.28
UL	34.21	40.38	1.34±0.31	1.40±0.30	1.18±0.29	1.26±0.34	0.12±0.16	0.15±0.19	0.17±0.23	0.22±0.26
Mean	**44.74**	**39.42**	**1.45±0.32**	**1.39±0.31**	**1.26±0.33**	**1.25±0.35**	**0.15±0.20**	**0.15±0.18**	**0.23±0.28**	**0.21±0.26**

nearly half of the proportion of this variation, 0.15±0.01 (46.88%), was due to within-population genetic variation (H_S). The genetic differentiation coefficient (G_{ST}) was 0.53 and mean gene flow (N_m) within a generation among the 8 populations was 0.45. Genetic distances ranged from 0.0707 to 0.4353 among population pairs. The minimum distance was between the ML and IL, while the maximum distance detected was between AK and ML (Table 3). The UPGMA dendrogram presented in Figure 2 shows that the eight populations are clustered under two distinct groups.

Six SRAP primer pairs generated a total of 52 well-resolved bands of which 100% were polymorphic in the 75 specimens sampled from 8 studied populations. The amplified fragments ranged in size from 350 bp to 1,500 bp. Genetic parameters for intra- and inter-population variability are given in Table 2. The percentage of polymorphic loci (*P*) in populations varied between 17.31% (AL) and 55.77% (MR) with an overall mean of 39.42%. The overall mean number of observed alleles per locus (n_a) was 1.39±0.31, while the overall mean number of effective alleles per locus (n_e) was 1.25±0.35. AL had the lowest value for n_a (1.17±0.25), and MR had the highest value (1.56±0.35). n_e values of the populations varied between 1.13±0.33 (AL) and 1.35±0.38 (AK). The overall means of Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.15±0.18 and 0.21±0.26, respectively. *h* values ranged from 0.07±0.11 in AL to 0.20±0.23 in AK, and *I* values ranged from 0.10±0.18 to 0.29±0.33 in the same populations. Total genetic variation (H_T) was 0.30±0.02. A half of the proportion of this variation, 0.15±0.01 (50.00%), was due to within-population genetic variation (H_S). The genetic differentiation coefficient (G_{ST}) was 0.52 and mean gene flow (N_m) within a generation among the 8 populations was 0.47. Genetic distances between the populations ranged from 0.059 to 0.415. The minimum distance detected was between the ML and IL, while the maximum distance was between MR and UL (Table 3).

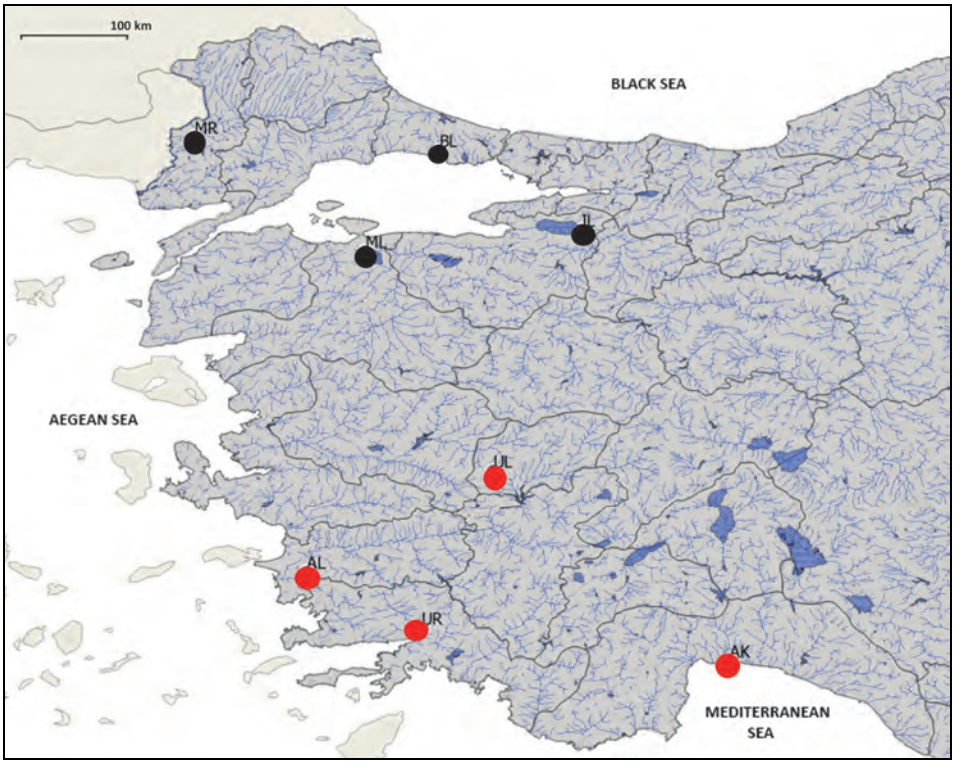

Figure 1. Water bodies where Gibel Carp (*Carassius gibelio*) was sampled in western Turkey. See Table 1 for site abbreviations. The two population groups as revealed by the UPGMA dendrograms are shown indifferent colours (black vs. red).

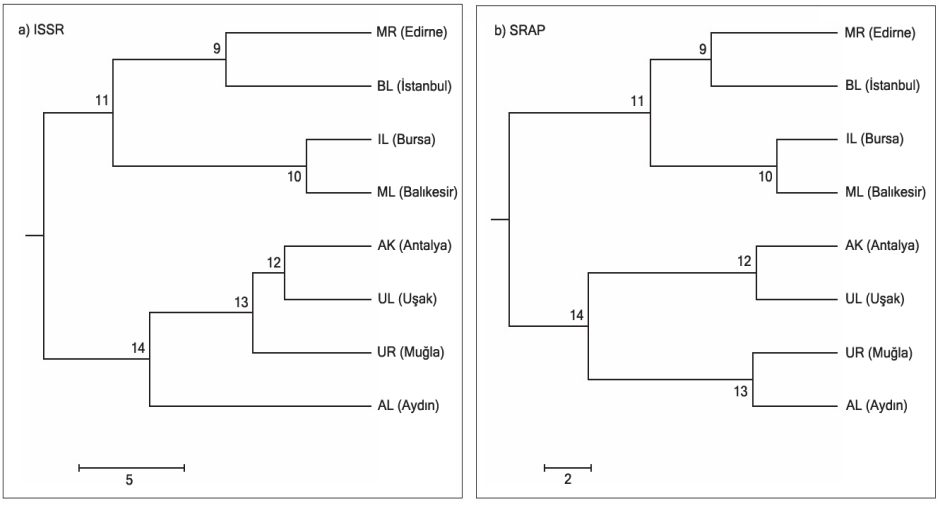

Figure 2. UPGMA dendrograms based on Nei's (1972) genetic distances among Gibel Carp (*Carassius gibelio*) populations in western Turkey.

Table 3. Estimates of Nei's (1972) genetic distances revealed by ISSR (below diagonal) and SRAP (above diagonal) markers among Gibel Carp (*Carassius gibelio*) populations.

	AK	AL	ML	IL	MR	BL	UR	UL
AK	***	0.2471	0.2786	0.3171	0.3209	0.3368	0.2146	0.0810
AL	0.2491	***	0.2268	0.2695	0.2758	0.3222	0.0844	0.2419
ML	0.4353	0.3300	***	0.0590	0.1896	0.1400	0.1949	0.2780
IL	0.3750	0.3082	0.0707	***	0.1966	0.1537	0.2145	0.3218
MR	0.2283	0.3345	0.3014	0.2292	***	0.1193	0.2793	0.4150
BL	0.2830	0.4176	0.3018	0.1902	0.1486	***	0.2645	0.3817
UR	0.1325	0.1502	0.2974	0.2753	0.2631	0.3368	***	0.2031
UL	0.0901	0.2747	0.4093	0.3328	0.2411	0.4176	0.1149	***

Discussion

The level of genetic diversity specifies the probability of a population to establish in new environments (Punnett, 1930). Studies on genetic structure of both native and non-native populations predict the baseline for understanding evolution during the establishment period. Although previous molecular studies in Gibel Carp populations have focused on resolving taxonomic issues, particularly in distinguishing and defining the geographic distributions of this species, recent studies have shifted to determine genetic variation of native and/or invasive populations. Indeed, genetic diversity in native and non-native Gibel Carp populations have been widely reported (e.g. Li & Gui, 2008; Keskin, Ağdamar, & Tarkan, 2013; Ağdamar & Tarkan, 2019). However, little information relevant to the population genetics of Gibel Carp (mainly on mitochondrial DNA) has been available from the eastern Mediterranean basin (Kalous, Bohlen, Rylková & Petrtýl, 2012; Keskin, Ağdamar, & Tarkan, 2013; Knytl, Kalous, Symonová, Rylková, & Ráb 2013; Geiger et al., 2014; Ribeiro, Rylková, Moreno-Valcárcel, Carrapato, & Kalous, 2015; Ağdamar & Tarkan, 2019).

In our study, tested ISSR and SRAP marker sets suggested genetically well-differentiated Gibel Carp populations with a low level of gene flow between the populations. High level of G_{ST} implies a considerable degree of differentiation among populations and low level of N_m is a low migration rate between population (Xiao & Gong, 2006). Our results suggesting high level intra-population genetic differentiation in Gibel Carp in Turkey are consistent with the findings of previous studies using various molecular markers (e.g. Ağdamar & Tarkan, 2019). Ji et al. (2014) used SRAP markers to compare genetic diversity and differentiation among three natural populations, a genetically selected strain and a cultured population of blunt snout bream (*Megalobrama amblycephala*) in China, and found F_{ST} (an analogue of G_{ST}) between 0.351 to 0.685. Similarly, Pechsiri & Vanichanon (2015) determined high genetic differentiation (G_{ST} = 0.28) with low gene flow (N_m = 1.28) with RAPD markers among three Slender Walking Catfish (*Clarias nieuhofii*) populations in Thailand. Abdul Muneer et al. (2009) reported similar results (G_{ST} = 0.506, N_m = 0.488) with RAPD markers on three yellow catfish (*Horabagrus brachysoma*) populations in South India river systems. The physical barrier is thought to be primary reason to inhibit the migration of populations and inter-breeding as for Gibel Carp populations examined in the present study.

Our results showed almost half of the genetic variation was within the examined populations (resp. 46.88 and 50.00%) while the rest was between the populations. This

relatively high level of inter-population variation in relation to intra-population variation suggest a high level of genetic differentiation and a low rate of gene flow between populations. We also found high genetic distances between the various Gibel Carp populations ranging from 0.059 to 0.415. This can be attributed to several colonization events of Gibel Carp from the different sources within a region, as was the case for *Lepomis gibbosus* in Portugal, which showed high levels of genetic differentiation based on genetic distance in eight reservoirs (Bhagat, Wilson, Fox & Ferreira, 2011). This could be supported by the fact that Gibel Carp have been widely introduced intentionally by local fisherman at regional scale and accidentally by government-based stocking practices of *Cyprinus carpio* at the country scale (Tarkan et al., 2015).

The percentage of polymorphism (*P*) at intra-population level in Gibel Carp for ISSR and SRAP markers was relatively high for some populations (Table 2), although in overall population the percentage was similar (resp. 44.74 and 39.42%) compared to other species. The fluctuant percentage polymorphism scored with both ISSR and SRAP markers in the present study may probably due to preferential amplification of non-coding iterative regions of the genome that may avoid natural selection (Callejas & Ochando, 2002).

Genetic relationship between the populations of studied suggest that there were two independent introduction events, one in the Marmara Region and the other in SW Turkey. After entering Turkey, Gibel Carp has most likely been accidentally introduced across the country through Common Carp (*Cyprinus carpio*) stockings. This was done mainly by various aquaculture facilities; the largest ones are situated in Edirne (Thrace) and Antalya (southern Turkey). Although none of our samples were obtained from these facilities, two of our sampling sites (Antalya - AK, and Edirne - MR) were very close to these facilities. We suggest that the source of studied Gibel Carp populations might be mainly from these two large facilities.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

References

Abdul Muneer, P. M., Gopalakrishnan, A., Musammilu, K. K., Mohindra, V., Lal, K. K., Basheer, V. S., & Lakra, W.S. (2009). Genetic variation and population structure of endemic yellow catfish, *Horabagrus brachysoma* (Bagridae) among three populations of Western Ghat region using RAPD and microsatellite markers. *Molecular Biology Reports, 36*, 1779–1791.

Ağdamar, S., & Tarkan, A. S. (2019). High genetic diversity in an invasive freshwater fish species, *Carassius gibelio*, suggests establishment success at the frontier between native and invasive ranges. *Zoologischer Anzeiger, 283*, 192–200.

Baysal, Ö., Siragusa, M., Ikten H., Polat, I., Gümrükcü, E., Yiğit, F., Carimi, F. & Teixeira da Silva, J. A. (2009). *Fusarium oxysporum* f. sp. *lycopersici* races and their genetic discrimination by molecular markers in West Mediterranean region of Turkey. *Physiological and Molecular Plant Pathology*, *74*, 68–75.

Baysal, Ö., Mercati, F., Ikten, H., Yıldız, R. Ç., Carimi, F., Aysan, Y., & Teixeira da Silva, J. A. (2011). *Clavibacter michiganensis* subsp. *michiganesis*: Tracking strains using their genetic differentiations by ISSR markers in Southern Turkey. *Physiological and Molecular Plant Pathology*, *75*, 113–119.

Bhagat, Y., Wilson, C. C., Fox, M, G., & Ferreira, M. T. (2011). Genetic relationships among pumpkinseed (*Lepomis gibbosus*) ecomorphs in freshwater reservoirs of Portugal. *Ecology of Freshwater Fish*, *20*, 287–298.

Callejas, C., & Ochando, M. D. (2002). Phylogenetic relationships among Spanish *Barbus* species (Pisces, Cyprinidae) shown by RAPD markers. *Heredity*, *89*, 36–43

Devran, Z., & Baysal, Ö. (2012). Genetic characterization of *Meloidogyne incognita* isolates from Turkey using sequence-related amplified polymorphism (SRAP). *Biologia*, *67*, 535–539.

Geiger, M.F., Herder, F., Monaghan, M.T., Almada, V., Barbieri, R., …. & Freyhof, J. (2014). Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources*, *14*, 1210–1221.

Gozlan, R. E., & Newton, A. C. (2009). Biological invasions: Benefits versus risks. *Science*, *324*, 1015.

Gozlan, R. E. Andreou, D., Asaeda, T., Beyer, K., Bouhadad, R., Burnard, D., …. & Britton, J. R. (2010). Pan-continental invasion of *Pseudorasbora parva*: towards a better understand freshwater fish invasions. *Fish and Fisheries*, 11, 315–340.

Harrison, J. S., & Mondor, E. B. (2011). Evidence for an invasive aphid "superclone": Extremely low genetic diversity in oleander aphid (*Aphis nerii*) populations in the Southern United States. *PLoS ONE*, *6*, e17524.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313–321.

Ji, W., Zhang, G. R., Ran, W., Gardner, J. P. A., Wei, K. J., Wang, W. M., & Zou, G. W. (2014). Genetic diversity of and differentiation among five populations of blunt snout bream (*Megalobrama amblycephala*) Revealed by SRAP markers: Implications for conservation and management. *PLoS ONE*, *9*, e108967.

Kalous, L., Bohlen, J., Rylková, K., & Petrtýl, M. (2012). Hidden diversity within the Prussian carp and designation of a neotype for *Carassius gibelio* (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, *23*, 11–18.

Keskin, E., & Atar, H. H. (2012). Molecular identification of fish species from surimi-based products labeled as Alaskan pollock. *Journal of Applied Ichthyology*, *28*, 811–814.

Keskin, E., Ağdamar, S., & Tarkan, A. S. (2013). DNA barcoding common non-native freshwater fish species in Turkey: Low genetic diversity but high population structuring. *Mitochondrial DNA*, *24*, 276–287.

Kimura, M., & Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*, *49*, 725–738.

Knytl, M., Kalous, L., Symonová, R., Rylková, K., & Ráb, P. (2013). Chromosome studies of european cyprinidfishes: cross-species painting reveals natural allotetraploid origin of a *Carassius* female with 206 chromosomes. *Cytogenetic and Genome Research*, *139*, 276–283.

Leprieur, F., Brosse, S., García-Berthou, E., Oberdorff, T., Olden, J. D., & Townsend, C. R. (2009). Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes. *Fish and Fisheries*, *10*, 88–97.

Lewontin, R. C. (1972). The apportionment of human diversity. *Evolutionary Biology*, *6*, 381–398.

Li, G., & Quiros, C. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics*, 103, 455–461.

Li, G., Gao, M., Yang, B., & Quiros, C. F. (2003). Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. *Theoretical and Applied Genetics, 107*, 168–180.

Li, F. B., & Gui, J. F. (2008). Clonal diversity and genealogical relationships of gibel carp in four hatcheries. *Animal Genetics, 39*, 28–33.

Moghaieb, R. E. A., Abdelhadi, A. A., El-Sadawy, H. A., Allam N. A. T., Baiome B. A., & Soliman M. H. (2017). Molecular identification and genetic diversity among *Photorhabdus* and *Xenorhabdus* isolates. *3 Biotech*, 7, 6.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist, 106*, 283–292.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, 70*, 3321–3323.

Pechsiri, J., & Vanichanon, A. (2015). Genetic diversity in slender walking catfish (*Clarias nieuhofii*) populations: Implications for population management. *Walailak Journal of Science and Technology, 13*, 511–519.

Punnett, R. (1930). The genetical theory of natural selection. *Nature, 126*, 595–597.

Ribeiro, F., Rylková, K., Moreno-Valcárcel, R., Carrapato, C., & Kalous, L. (2015). Prussian carp *Carassius gibelio*: a silent invader arriving to the Iberian Peninsula. *Aquatic Ecology, 49*, 99–104.

Robarts, D. W. H., & Wolfe, A. D. (2014), Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. *Applications in Plant Sciences, 2*, 1400017.

Tarkan, A. S., Gaygusuz, O., Gürsoy Gaygusuz, C., Saç, G., & Copp, G. H. (2012). Circumstantial evidence of gibel carp, *Carassius gibelio*, reproductive competition exerted on native fish species in a mesotrophic reservoir. *Fisheries Management and Ecology, 19*, 167–177.

Tarkan, A. S., & Marr, S. M. (2015). Non-native and translocated freshwater fish species in Turkey. *Fishes in Mediterranean Environments, 3*, 1–28.

Townsend, C. R. (2003). Individual, population, community, and ecosystem consequences of a fish invader in New Zealand streams. *Conservation Biology, 17*, 38–47.

Xiao, L. Q., & Gong, X. (2006). Genetic differentiation and relationships of populations in the *Cycas balansae* complex (Cycadaceae) and its conservation implications. *Annals of Botany, 97*, 807–812.

Yeh, F. C., Boyle, T., Rongcai, Y., Ye, Z., & Xiyan J. M. (1999). *Popgene version 1.32 Microsoft Windows-Based freeware for population genetic analysis*. Edmonton (Canada): University of Alberta.

Yerli, S. V., Mangıt, F., Emiroğlu, Ö., Yeğen, V., Uysal, R., Ünlü, E., … & Zengin, M. (2014). Distribution of invasive *Carassius gibelio* (Bloch, 1782) (Teleostei: Cyprinidae) in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences, 14*, 581–590.