

Article

Low Genetic and Parasite Diversity of Invasive Pumpkinseed *Lepomis gibbosus* (Centrarchidae) Expanding in Türkiye

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Abstract: Multiple factors can facilitate invasion success, with the absence of natural enemies, such as predators and parasites, recognised as conferring a significant advantage on invasive over native species. Pumpkinseed *Lepomis gibbosus* (Centrarchidae) represents one of the most successful freshwater fish invaders in Europe. Previous research has highlighted genetic differences between pumpkinseed populations in Türkiye and those in other European regions, attributed to rapid adaptation to new environmental conditions. This study aimed to investigate whether these highly adapted pumpkinseed populations in Türkiye benefit from a potential release from parasites, as proposed by the enemy-release hypothesis. Genetic characterisation of pumpkinseed populations from both European and Asian parts of Türkiye revealed that they share the same cytochrome *c* oxidase I haplotype as European populations. Microsatellite analysis indicated low genetic diversity, with STRUCTURE analysis confirming the clustering of all Turkish populations, suggesting a common source. Consistent with the low genetic diversity indicative of a small founding population, we observed a limited number of co-introduced parasite species, including the myxozoan *Myxobolus dechtiari*, the monogenean *Onchocleidus dispar*, and the digenean *Posthodiplostomum centrarchi*. Parasite infection by local parasites acquired in Türkiye was rare. Parasite diversity, species richness, and equitability were low, with only nine parasite taxa identified in all four pumpkinseed populations. The most diverse parasite community was found in Değirmenköy Reservoir, located in the European part of Türkiye, where seven parasite taxa were identified. While our study did not uncover genetically distinct pumpkinseed populations in Türkiye, the fish demonstrated resilience against most local parasite species, potentially providing them with an advantage over native species, aligning with the enemy-release hypothesis.

Keywords: microsatellites; cytochrome *c* oxidase I; parasitism; species introductions; Türkiye

1. Introduction

Fish play a crucial role in the economies of many countries, yet risk management measures, such as quarantine controls, are often less stringent than for other animals [1].



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The incidence of freshwater fish introductions has surged in recent decades due to the expansion of global trade, and this trend is expected to persist in the coming years [2,3]. The unintentional release or escape of introduced fish into natural habitats, resulting in invasive populations that disrupt native ecosystems, serves as a prime example of the socio-economic, ecological, and evolutionary impacts of globalisation on local biodiversity [4–7]. The adverse effects of non-native species on indigenous fauna encompass predation, competition for food and space, hybridisation, alteration of food webs and biogeochemical cycles, as well as the transmission of parasites and pathogens [4,8]. In particular, non-native fish parasites are a global problem affecting aquaculture and natural ecosystems worldwide [9,10].

Pumpkinseed (*Lepomis gibbosus* (L., 1758), Actinopterygii: Centrarchidae) is a species indigenous to freshwater habitats in eastern North America, ranging from New Brunswick, Canada, to north-eastern Georgia, USA [11]. Initially introduced to Europe as an ornamental fish in 1877, transported from Canada to Paris [12], the species was subsequently released into open waters, primarily in southwestern France, where stable populations were established around 1900 [13,14]. Over the following century, the pumpkinseed was introduced to nearly all European countries and has recently become an invasive species across much of the continent, spanning from the Iberian Peninsula in the west to coastal rivers of the Sea of Azov in the east [15,16]. In Türkiye, the fish was first documented in the Ipsala Canal (Meriç River drainage) in 1982 [17]. In the Asian part of Türkiye, it was initially observed in the Sarıçay and Dipsiz-Çine streams (Muğla, Anatolia) between 1999 and 2001 [18–20], subsequently spreading throughout the Aegean Region of Anatolia [21–26]. More recently, the fish has expanded into numerous new habitats in the Marmara and Aegean regions, encompassing both the European and Asian parts of Türkiye [27,28]. Currently, Anatolia represents the sole Asian region where the invasive pumpkinseed is established. However, other Asian countries, such as China, Iran, Japan, and South Korea, have experienced introductions of another invasive centrarchid fish, the bluegill (*Lepomis macrochirus* Rafinesque, 1819), in the past [29–32].

Molecular genetic studies of invasive species offer valuable insights into their characteristics, evolutionary processes, and genetic adaptations that contribute to their successful establishment in new environments [33]. Such analyses also aid in elucidating the origin and invasion history of these species [34]. European populations of pumpkinseed have been extensively investigated using both mitochondrial [21,35–37] and nuclear [38] gene markers, revealing generally low genetic variability. In contrast to other European populations, where all pumpkinseed populations are associated with a single haplotype of cytochrome *c* oxidase I (*cox1*) mitochondrial DNA [35,36], higher *cox1* diversity has been observed in Türkiye [21,39]. The three *cox1* haplotypes identified in Türkiye differ from, yet are derived from, the common haplotype found in other European and North American pumpkinseed populations, suggesting the species' evolutionary adaptability under the influence of different selection pressures, such as environmental factors [21].

In addition to abiotic environmental factors like temperature or salinity, non-native species face pressure from biotic factors such as parasitism. The role of parasites in the success of invasive species has gained widespread recognition, with parasites significantly influencing interactions between non-native and native species [40]. Non-native species may introduce new parasite species into the invaded ecosystems, which could be specific to their original hosts, as well as the agents of diseases for the local fauna [41]. Release from pressure by natural parasites advantaging non-native species over natives is among the most commonly proposed hypotheses regarding the role of parasites in biological invasions [42]. There has been an increased interest in the investigation of pumpkinseed parasites in various non-native European populations in Europe during the recent decades, revealing considerable variation in parasite abundance and diversity across different geographical regions, time since introduction, and host genetic diversity [36,38,43,44]. Studies on parasite communities have identified a range of North American parasite species co-introduced to Europe with pumpkinseed, including myxozoans [45], centrarchid-specific

monogeneans [38,46–48], cestodes [49], and trematodes [50,51]. However, these studies have predominantly focused on pumpkinseed populations of the common *cox1* haplotype.

Therefore, the main objective of this study was to investigate parasite communities of pumpkinseed in Türkiye, where different haplotypes and high environmental tolerances of this fish species have recently been observed [21]. We expected that genetically reduced pumpkinseed populations derived from the common haplotype found in other European populations indicating the rapid evolutionary adaptability to new environmental conditions [21] would exhibit low parasite diversity and abundance, especially regarding co-introduced parasite species [38,52]. Reduced genetic diversity related to small founder populations may be associated with a decreased probability of parasite transmission to new areas due to their absence in the founding population or presence at such low densities that the parasite population cannot be sustained [52]. To confirm the genetic variability of fish collected in Türkiye, we conducted a molecular analysis using both mitochondrial (*cox1*) and nuclear (microsatellites) markers, comparing the obtained results with those of fish collected in various European localities as published by Ondračková et al. [36,38]. This study also presents the first data on parasite communities in pumpkinseed that were introduced to Asia.

2. Materials and Methods

2.1. Fish Sampling

The fish were sampled from four locations in Türkiye in October 2021 (Figure 1). Two of these locations were situated in the Aegean region of Anatolia. These locations were Sarıçay River (37.316111, 27.706667) and Dipsiz-Çine Stream (37.763889, 27.833611). One location was in north-western Anatolia—Davuldere Reservoir (40.907186, 30.039250), and one was in the European part of the City of Istanbul—Değirmenköy Reservoir (41.166692, 28.017958). According to Ağdamar et al. [21], fish from Davuldere Reservoir corresponded to *cox1* haplotype H_2 and those from Sarıçay River to H_3, while fish from Dipsiz-Çine Stream and Değirmenköy Reservoir had not been genotyped previously.

Electrofishing using backpack electrofishing gear (SAMUS 725G and SAMUS 1000) was the preferred method for fish sampling for parasitological investigation [53]. The fish were transported alive in aerated river water to the laboratory, where they were placed into aerated tanks with water from the sampling sites. All fish were dissected within three days of sampling to ensure maximum parasite recovery (see [54]). Before dissection, the standard length (SL, mm), total length (TL, mm), total weight (W, g), and eviscerated weight (Wb, g) were measured for each fish (Table 1), and a sample of the caudal fin was preserved in 96% ethanol for further molecular analysis.

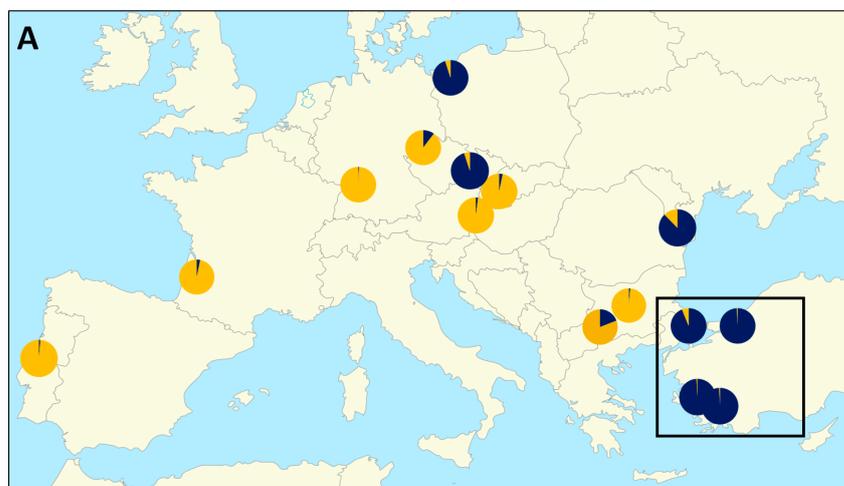


Figure 1. Cont.

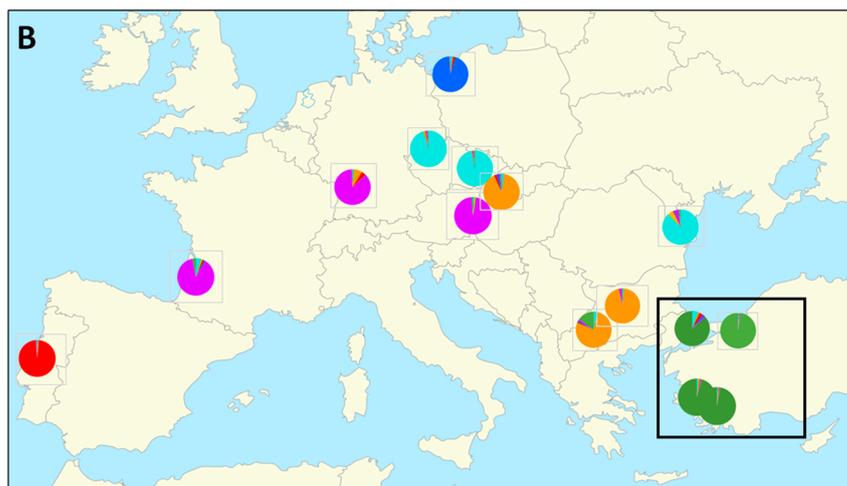


Figure 1. Map indicating the populations of pumpkinseed *Lepomis gibbosus* compared in this study. Populations from Türkiye are marked by black frames. Based on the STRUCTURE analysis, dividing into two (A) and six (B) clusters (marked by different colours) was identified as the best-supported model of population structuring.

Table 1. Size and weight of the sampled fish in Türkiye. SL—standard length, TL—total length (in mm), W—total weight, We—eviscerated weight (in g).

Locality		SL, mm	TL, mm	W, g	We, g
Sarıçay River (n = 21)	M ± sd	70.4 ± 4.8	86.2 ± 5.9	11.4 ± 2.6	11.0 ± 2.6
	Min–max	61.4–77.4	77.3–97.8	7.5–16.7	7.3–16.3
Dipsiz-Çine Stream (n = 20)	M ± sd	48.3 ± 21.8	59.0 ± 26.3	5.9 ± 11.5	5.7 ± 11.2
	Min–max	28.6–110.3	35.8–133.9	0.7–41.1	0.6–39.9
Davuldere Reservoir (n = 20)	M ± sd	48.1 ± 6.8	59.4 ± 8.1	2.9 ± 2.0	2.8 ± 2.0
	Min–max	42.7–74.3	52.1–90.4	1.8–11.3	1.6–11.2
Değirmenköy Reservoir (n = 20)	M ± sd	57.4 ± 19.4	70.4 ± 24.2	7.6 ± 9.4	7.3 ± 8.9
	Min–max	40.6–98.4	50.1–119.7	1.6–29.2	1.6–28.0

2.2. Genetic Analysis of Fish

The DNA of the fish was extracted using the commercial GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA USA) following the enclosed instructions. For 31 fish, the partial mitochondrial *cox1* gene was amplified using the primers Fish-F1 (5′TCAACCAACCACAAAGACATTGGCAC3′) and Fish-R1 (5′TAGACTTCTGGGTGGCCAAAGAATCA3′) [55] with the PCR Multiplex Kit (Qiagen, Hilden, Germany) under standard protocol and an annealing temperature of 58 °C. The PCR products were commercially sequenced at Eurofins Genomics Germany GmbH and analysed using Geneious v.9.0.5. software (<http://www.geneious.com>). Samples from 81 fish individuals from the four localities were genotyped for five polymorphic microsatellite loci (Lmar9, Lmar18, Lmar29, RB7, and RB20) with the PCR details described by Ondračková et al. [38]. The PCR products were electrophoresed on the ABI Prism®3130 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) and analysed using Geneious.

Genetic divergence among populations was estimated by pairwise F_{ST} [56] using 1000 permutations in GENETIX 4.05 [57]. The Hardy–Weinberg equilibrium (HWE) test was performed for each locus in all populations using the Markov chain method (“Exact probability test”) in Genepop v.4.7 [58]. The number of different alleles (NA), the Shannon information index (I), expected heterozygosity (He), unbiased expected heterozygosity (UHe), observed heterozygosity (Ho), fixation index (F), and percentage of polymorphic loci (P) were all calculated in GenAlEx v. 6.51 [59,60]. Newly generated sequences of one specimen per locality were deposited in the GenBank database under accession numbers PP469740–PP469743.

The population genetic structure was analysed using the Bayesian clustering algorithm implemented in the program STRUCTURE v. 2.3.4 [61]. The program was run with 10 independent simulations for each K from 1 to 10, with 1 million iterations for each simulation (burn-in = 100,000 iterations) using an admixture model and a correlated allele frequencies model ($\lambda = 1$). The output of the STRUCTURE analysis was post-processed using CLUMPAK software v. 1.1 [62] to identify separate groups of runs based on similarity between Q-matrices for each K using the LargeKGreedy algorithm, random input order, and 2000 repeats. Different modes were identified for similarity scores from the 20 runs for each K value at a threshold of 0.9. The web-based software STRUCTURE HARVESTER v. 0.7 July 2022 [63] was used to summarise output data from STRUCTURE. The likelihood of K ($\ln \Pr(X|K)$), the ΔK criterion using the method of Evanno et al. [64], and a proportion of similar runs that formed the major modes for each K were used to infer the best number of real populations in the datasets.

2.3. Parasite Collection, Processing and Analysis

A total of 81 individual fish were examined for parasites (Table 1). The fins, skin, gills, muscles, and internal organs were inspected for parasites. Myxozoans were examined live using light microscopy and photographed for further identification. Monogeneans were preserved in GAP (glycerine-ammonium-picrate) and prepared as semi-permanent slides [65] while digeneans and nematodes were preserved in hot 4% formaldehyde, with digeneans stained with iron acetic carmine, dehydrated in ethanol of increasing concentration, and mounted in Canada balsam as permanent slides [66,67]. Crustaceans were preserved in 4% formaldehyde and identified under light microscopy. All parasites were identified based on their morphological features at the species level where possible or at the highest taxonomic level when species-level identification was impossible. A subsample of larval digeneans and nematodes, where morphological identification is difficult, was preserved in 96% ethanol for further molecular analysis.

The DNA of parasites (larval digeneans and nematodes) was extracted using the Invisorb[®] Spin Forensic Kit (STRATEC Molecular, Berlin, Germany) following the standard protocol. We used forward primer BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and the reverse primer 4S (5'-TCTAGATGCGTTCGAA(G/A)TGTCGATG-3') [68] to obtain the ITS1 partial sequence for *Posthodiplostomum*, with an annealing temperature of 58 °C (PCR details in [43]). For obtaining the 18S partial sequence for nematodes, we employed the forward primer 18S-CL-F3 (5'-CTTGCTCAAAGATTAAGCCATGCAT-3') [69] and the reverse primer D2AR (5'-ACTTTCCTCACGGTACTTGT-3') (reverse of D2A from [70]). The reaction mix contained 4 µL of extracted DNA, 0.3 µL of each primer (10 µM), 2 µL of buffer A, 0.2 µL of dNTPs (10 mM), 0.2 µL of MgCl₂ (25 mM), 0.5 U/µL of Taq polymerase, and ddH₂O up to a total volume of 10 µL. For PCR analysis, we used the KAPA2G Robust HotStart PCR Kit (Kapabiosystems, Wilmington, MA, USA), with amplification conducted in a Mastercycler ep gradient S thermocycler (Eppendorf, Hamburg, Germany) following a temperature profile of the PCR reaction of 95 °C for 2 min, 5 cycles of (94 °C for 30 s, 45 °C for 40 s, 72 °C for 2 min), 40 cycles of (94 °C for 30 s, 58 °C for 40 s, 72 °C for 2 min), and a final extension at 72 °C for 5 min. All PCR products were purified using an ExoSAP-IT kit (Affymetrix Inc., Santa Clara, CA, USA) per the manufacturer's protocol. Subsequently, the PCR products were commercially sequenced at Eurofins Genomics Germany GmbH. The sequences were checked and aligned using Geneious, and the newly generated sequences were compared with the NCBI database using BLASTn to assess sequence similarity.

Prevalence, intensity of infection, and abundance were calculated following Bush et al. [71]. Mean abundance was expressed as the mean number of parasites for all hosts in a sample, intensity of infection as the mean number of parasites for infected hosts, and prevalence as the percentage of infected hosts. The parasite community was analysed at both the infracommunity (IC, including all parasites on a single host) and component community (CC; all parasites in a host population) levels [71]. Similarity in parasite communities was evaluated based on qualitative and quantitative data on parasite species

composition using the Sørensen and Bray–Curtis similarity indices, respectively. Diversity, describing the relationship of the ratios of all parasites in a host population or specimen, was calculated by the Shannon–Wiener index (H). Equitability (J) was used to measure the evenness with which individuals are distributed among the taxa present [72]. The trend for parasites to appear in the same IC was expressed by the infracommunity index (ICI). The ICI describes the frequency of double and multiple infections by a single parasite species in a distinct host [73]. Values > 0.4 indicate strongly interactive parasite species, >0.3 indicate a great tendency to join the community, while values > 0.2 suggest moderately interactive parasite species [73,74].

3. Results

3.1. Genetic Characterisation of Fish Host

All four pumpkinseed populations sampled in Türkiye exhibited a single *cox1* haplotype. The partial *cox1* sequences ranged between 630 and 636 bp in length and corresponded to those obtained from fish collected in other non-native European (GeneBank accession numbers KY231843, MW473462, HQ600737, KJ553720, MK439905) as well as native North American (KX145030, MF621725, KX145396, KX145175, KX145351, MF621724, MT667250, MF621726, JN026988) populations. A total of 19 alleles were observed for the five microsatellite loci, with four loci being polymorphic, while Lmar9 had a single allele in all four populations. All four populations were in Hardy–Weinberg equilibrium, and values of total and average allele richness, number of effective alleles, Shannon information index, and heterozygosity, as shown in Table 2, were similar among particular populations. Three private alleles were observed at each of the Davuldere and Değirmenköy Reservoirs.

Table 2. Results of testing of genetic variability for five microsatellite loci. N = No. of individuals; NA = number of different alleles, I = Shannon’s Information Index; Ho = Observed Heterozygosity; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity; F = Fixation Index; HWE = P-values of Hardy–Weinberg Equilibrium; P = Percentage of Polymorphic Loci.

Population	N	NA	I	Ho	He	Uhe	F	HWE	%P
Sarıçay River	21	2.400	0.579	0.333	0.362	0.370	0.074	0.940	80
Dipsiz-Çine Stream	20	2.000	0.434	0.280	0.281	0.288	−0.021	0.993	80
Davuldere Reservoir	20	2.600	0.572	0.330	0.310	0.318	−0.066	0.429	60
Değirmenköy Reservoir	20	2.400	0.658	0.450	0.427	0.438	−0.080	0.520	80

STRUCTURE analysis conducted using fish from four populations in Türkiye and 11 other European localities [38] suggested that K = 2 and 6 are the most supported models of population structuring, based on a combination of the likelihood of K ($\ln \Pr(X|K)$), the ΔK criterion [64], and the proportion of similar runs. For K = 2, the two clusters corresponded to (1) Türkiye (all four localities) + Kartal (Ukraine) + Jez (Czech Republic) + Gryfino Canal (Poland) and (2) localities in the basins of the rivers Danube (Austria, Bulgaria, Slovakia), Rhine (Germany), Elbe (Czech Republic), Gironne (France), and Sado (Portugal) (Figure 1A). For K = 6, all populations from Türkiye formed a separate cluster, while Değirmenköy Reservoir was partially mixed with other European populations (Figure 1B).

3.2. Composition of Parasite Communities

Nine parasite taxa were found to infect pumpkinseed at four sites in western Türkiye, including one myxozoan, one monogenean, one digenean, five nematodes, and one crustacean (Table 3). Six parasite taxa were in larval stages, including the metacercariae of a digenean and all nematodes, which were all endoparasites located in internal organs, mesentery, and muscles. Ectoparasites were represented by myxozoans and monogeneans infecting gills and a parasitic copepod found on the fins or penetrating the body cavity. Three species, namely myxozoan *Myxobolus dechtiari* Cone and Anderson, 1977, monoge-

nean *Onchocleidus dispar* (Mueller, 1936), and metacercariae of *Posthodiplostomum centrarchi* Hoffman, 1958, were introduced to Europe and are natural parasites of pumpkinseed in North America. These species had relatively high abundances and prevalence (Table 3). Identification of *P. centrarchi* was confirmed through both morphology and genetic analysis, with two sequences obtained from the Sarıçay River (Asia) and one from Değirmenköy Reservoir (Europe). One of the sequences from the Sarıçay River and the Değirmenköy Reservoir were identical and differed from the second sequence from the Sarıçay River with one substitution. All sequences showed 99.9–100% correspondence with *P. centrarchi* from pumpkinseed collected in the Hudson River in Canada (GeneBank Acc. No. MH521251), Hungary (MN080282), and in Portugal (MF171006; denoted as *P. cf. minimum*).

Table 3. Prevalence, mean abundance, species richness, mean infracommunity (IC) richness, Shannon and Equitability indices of parasite communities of the invasive pumpkinseed in four localities in Türkiye. **Bold:** greater part of homogeneity.

Locality	N	Prevalence (in %)	Mean Abundance	Species Richness	Mean IC (MI ± sd)	Shannon	Equitability
Sarıçay River	21	100	124.5	3	2.05 ± 0.65	0.25	0.23
Dipsiz-Çine Stream	20	25	0.5	5	0.30 ± 0.56	1.47	0.91
Davuldere Reservoir	20	70	2.5	2	0.75 ± 0.54	0.10	0.14
Değirmenköy Reservoir	20	90	8.8	7	1.70 ± 1.10	1.24	0.64

Out of all parasite taxa pumpkinseed acquired in Türkiye, only two nematodes were found in higher prevalence in Sarıçay River, namely larvae of *Eustrongylides* sp. (57% of fish infected) and larvae of *Parquimperia tenerrima* (von Linstow, 1878) Baylis, 1934 (48% of fish infected), with higher abundance (6.6) observed in the latter. Other taxa acquired in Türkiye (i.e., Aquariidae gen. sp., *Contracaecum* sp., Nematoda sp., *Lernaea cyprinacea* (Linnaeus, 1758)) occurred rarely. Larvae of *Eustrongylides* sp. Were found in all four localities. Both nematode species were subjected to genetic analysis. A sequence of 18S rDNA 775 bp long obtained from *P. tenerrima* (Sarıçay River) showed the highest similarity to *P. africana* (99.6%; difference by three substitutions) from *Anguilla marmorata* Quoy & Gaimard, 1824 in South Africa (JF803925). Larvae of *Eustrongylides* (18S rDNA, 849 bp) obtained from the Dipsiz-Çine Stream and Sarıçay River showed the highest similarity (with query cover > 80%) to *Eustrongylides* sp. (99.6 and 99.4%; difference caused by 1 substitution and one gap, and four unreadable bases, respectively) from *Alligator sinensis* Fauvel, 1879 in China (PP236907).

Maximum parasite prevalence (100%) was found in the Sarıçay River (Table 4). The IC species richness ranged from 0 to 4 per fish. All types of infracommunities were observed only in Değirmenköy Reservoir. Three species IC were found in the Sarıçay River, while two species IC were observed in the Dipsiz-Çine Stream and Davuldere Reservoir (Figure 2). Two parasite species occurred as strongly interactive in the parasite community, i.e., *O. dispar* in the Davuldere Reservoir and *P. centrarchi* in the Sarıçay River. Both species had a clear tendency to join the community in Değirmenköy Reservoir, together with larval nematodes Aquariidae gen. sp. In the Dipsiz-Çine Stream, all parasite taxa were moderately interactive (Table 5).

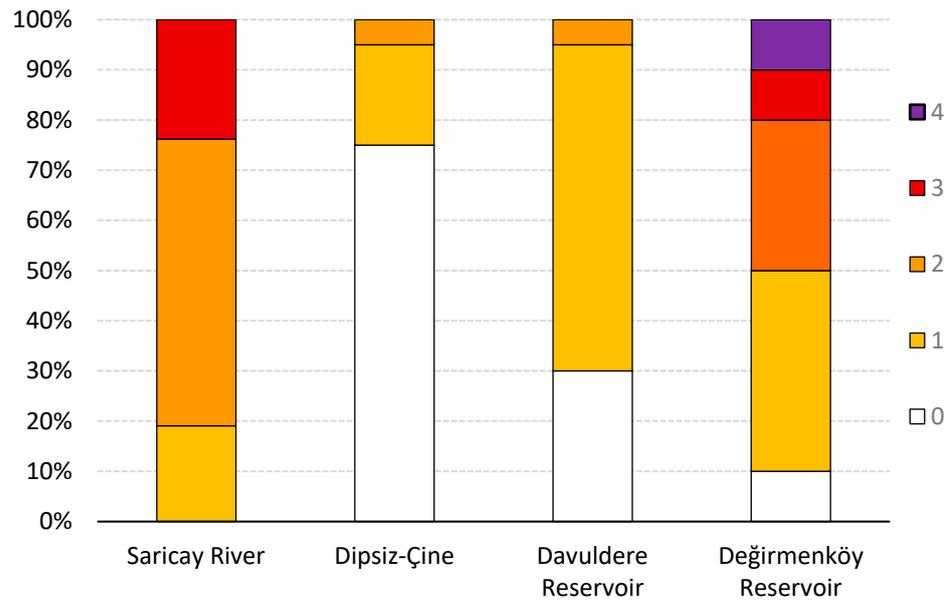


Figure 2. The representation of infracommunities (ranging from zero to four parasite taxa per fish individual) of the invasive pumpkinseed in different localities in Türkiye.

Table 4. Infection parameters of pumpkinseed *Lepomis gibbosus* from four localities in Türkiye: P—prevalence, in %; MI—mean intensity \pm standard deviation and minimum—maximum range; and A- mean abundance.

Parasite Species	Site	Indices	Sarıçay River	Dipsiz-Çine Stream	Davuldere Reservoir	Değirmenköy Reservoir
MYXOZOA						
<i>Myxobolus dechtiari</i>	Gills	P, %				15.0
		MI \pm sd (min-max)				15.3 \pm 8.1 (6-20)
		A				2.30
MONOGENEA						
<i>Onchocleidus dispar</i>	Gills	P, %			70.0	55.0
		MI \pm sd (min-max)			3.4 \pm 2.3 (1-8)	1.6 \pm 0.8 (1-3)
		A			2.40	0.90
DIGENEA						
<i>Posthodiplostomum centrarchi</i>	Mesentery, liver, spleen, heart, gonads, muscles, head, eyes, coelom	P, %	100.0	5.0		60.0
		MI \pm sd (min-max)	117.5 \pm 48.0 (48-223)	3.0 (3)		7.7 \pm 11.4 (1-41)
		A	117.5	0.15		4.60
NEMATODA						
<i>Eustrongylides</i> sp.	Mesentery	P, %	57.1	5.0	5.0	5.0
		MI \pm sd (min-max)	1.6 \pm 0.7 (1-3)	1.0 (1)	1.0 (1)	1.0 (1)
		A	0.9	0.05	0.05	0.05
<i>Contracaecum</i> sp.	Mesentery	P, %		10.0		10.0
		MI \pm sd (min-max)		2.0 \pm 1.4 (1-3)		1.0 (1)
		A		0.2		0.1
<i>Paraquimperia tenerrima</i>	Mesentery	P, %	47.6			
		MI \pm sd (min-max)	13.9 \pm 21.8 (1-70)			
		A	6.6			

Table 4. Cont.

Parasite Species	Site	Indices	Sarıçay River	Dipsiz-Çine Stream	Davuldere Reservoir	Değirmenköy Reservoir
Aquariidae gen. sp.	Intestine wall, mesentery	P, %				20.0
		MI ± sd (min–max)				3.8 ± 2.4 (1–7)
		A				0.75
Nematoda gen. sp.	Liver	P, %		5.0		
		MI ± sd (min–max)		1.0 (1)		
		A		0.05		
COPEPODA <i>Lernaea cyprinacea</i>	Fins, mesentery	P, %		5.0		5.0
		MI ± sd (min–max)		1.0 (1)		1.0 (1)
		A		0.05		0.05

Table 5. Infracommunity index of parasite species infecting the invasive pumpkinseed in four localities in Türkiye. **Bold:** parasite species with great tendency to join the infracommunity.

Parasite Species	Sarıçay River	Dipsiz-Çine Stream	Davuldere Reservoir	Değirmenköy Reservoir
<i>Myxobolus dechtiari</i>				0.09
<i>Onchocleidus dispar</i>			0.93	0.32
<i>Posthodiplostomum centrarchi</i>	0.49	0.17		0.35
<i>Eustrongylides</i> sp.	0.28	0.17	0.07	0.03
<i>Contracaecum</i> sp.		0.33		0.09
<i>Paraquimperia tenerrima</i>	0.23			
Aquariidae gen. sp.				0.31
Nematoda gen. sp.		0.17		
<i>Lernaea cyprinacea</i>		0.17		0.03

3.3. Parasite Diversity

The parasite species richness ranged from two (Davuldere Reservoir) to seven taxa (Değirmenköy Reservoir) (Table 3). High Shannon–Weiner diversity ($H = 1.47$) and equitability of the parasite community ($J = 0.91$) were found in the Dipsiz-Çine Stream; nevertheless, most of the fish (75%) at this locality were uninfected, and infected fish were parasitised with low intensities. Low diversity was observed in the Davuldere Reservoir and Sarıçay River ($H < 0.25$), with these 2 locations demonstrating the lowest equitability values ($J = 0.14$ and 0.23 , respectively).

3.4. Similarity in Parasite Communities

Qualitative and quantitative similarities between parasite communities of pumpkinseed from particular localities are presented in Table 6. The highest qualitative similarity in parasite communities measured by the Sørensen index was observed between Dipsiz-Çine Stream and 2 other localities, Değirmenköy Reservoir (67%) and Sarıçay River (50%). Quantitative similarity measured by the Bray–Curtis index was generally very low, with the highest values found between Davuldere Reservoir and Değirmenköy Reservoir (17%) and the minimum similarity found between Davuldere Reservoir and Dipsiz-Çine Stream (29% for Sørensen) and Davuldere Reservoir and Sarıçay River (0.1% for Bray–Curtis; Table 6, Figure 3).

Table 6. Matrix of qualitative (Sørensen index, below diagonal) and quantitative Bray–Curtis index (above diagonal) similarities between parasite communities of pumpkinseed *Lepomis gibbosus* sampled in four localities in Türkiye.

	Sarıçay River	Dipsiz-Çine Stream	Davuldere Reservoir	Değirmenköy Reservoir
Sarıçay River		0.003	0.001	0.067
Dipsiz-Çine	0.500		0.034	0.076
Davuldere Reservoir	0.400	0.286		0.170
Değirmenköy Reservoir	0.400	0.667	0.444	

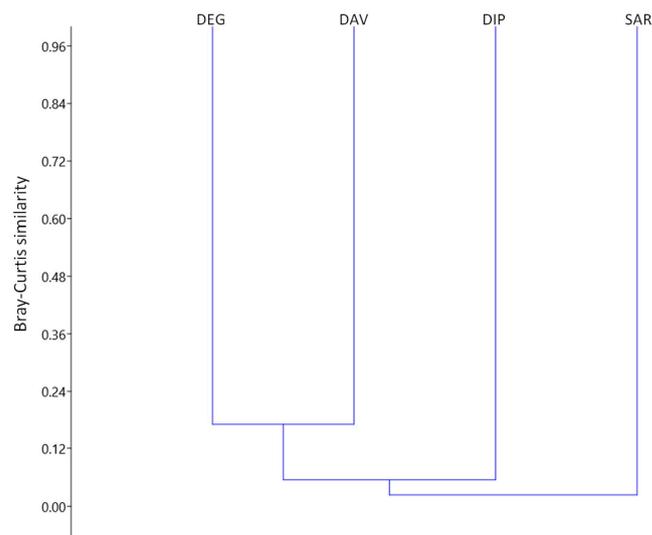


Figure 3. Dendrogram on Bray–Curtis similarity between parasite communities of *Lepomis gibbosus* at four localities in Turkey. DEG—Değirmenköy Reservoir, DAV—Davuldere Reservoir, DIP—Dipsiz-Çine Stream, SAR—Sarıçay River.

4. Discussion

Pumpkinseed collected at four localities in Türkiye exhibited low genetic variance in both mitochondrial and nuclear markers. All fish analysed were associated with a single *cox1* haplotype. The same haplotype was previously found in other non-native populations in Europe [21,35,36] and many native populations in North America [21]. Despite previous studies revealing two different haplotypes at Sarıçay River and Davuldere Reservoir [21,39], our study failed to find these at the same localities ten years later. Pumpkinseed dispersal through Türkiye since its first record in the Turkish Thrace Region [17] followed the pathways of carp stocking, with extensive fish production facilities in the western part of Türkiye being the most probable source [21], and subsequent spread facilitated by local fishermen [75]. However, the reason for our failure to collect fish with the previously detected haplotypes remains a question. One possible explanation might be associated with multiple introductions to the same water bodies with ongoing stockings in Türkiye. According to Ağdamar et al. [21], common carp-rearing ponds in Türkiye are often affected by seasonal floods from the Meriç River, which can lead to the invasion of non-native fish in nearby large fish production units. Similar unintentional introduction through transboundary river systems was reported for *Heteropneustes fossilis* (Bloch, 1794) in the Dicle–Fırat (Tigris–Euphrates) River system [76,77]. As pumpkinseed in Greece belongs to the common European *cox1* haplotype [35], it is possible that subsequent carp stockings unintentionally co-introduced pumpkinseed of this haplotype over ten years, leading to its dominance at the localities. Additionally, there may be natural changes in the occurrence and detection of haplotypes over time due to sampling efforts or stochastic processes [78].

Although new haplotypes were detected in 2015, they may have been present but not detected in 2021 owing to the smaller sampling size.

Results obtained from microsatellite data further supported the common origin of pumpkinseed populations in Türkiye and the rest of Europe. All four pumpkinseed populations collected in Türkiye clustered together, and their allelic composition appears to be most similar to fish from the lower Danube basin in Ukraine, i.e., a country located near Türkiye, and two populations in central Europe in the Czech Republic and Poland (Figure 1A). Unfortunately, our dataset did not include fish from Greece, which data might help confirm the primary source of pumpkinseed in Türkiye. Further successive separation of populations in the STRUCTURE analysis showed that Turkish populations grouped together and formed a separate cluster, indicating their strong genetic interrelationship (Figure 1B), which is in agreement with the suggestion of a single source for different localities in western Türkiye and Anatolia by Ağdamar et al. [21]. Interestingly, the population from Değirmenköy Reservoir, located in the European part of Istanbul, included specimens with different genetic origins, likely due to multiple introductions from various sources.

Based on the obtained results, the prediction that pumpkinseed populations with different haplotypes derived from a common European haplotype that is tolerant to a range of environmental conditions [21] would show low parasite diversity and abundance cannot be fully assessed. However, despite collecting fish of the common European haplotype, their parasite diversity appeared to be low in terms of parasites co-introduced along with the fish host and those acquired in the new environment. Similar results were found in pumpkinseed populations from Portugal and the Czech Republic, which exhibited low microsatellite allelic richness and heterozygosity [38], comparable to those in Türkiye. According to Luikart et al. [79], reduced heterozygosity may lead to increased parasite load in wild populations. However, this was not a general trend observed in our study, as most localities were inhabited by pumpkinseed with extremely low parasite abundance. Despite low heterozygosity and allelic richness, pumpkinseeds were rarely infected with local parasites. Moreover, except for the Sarıçay River, the infection by North American parasites was not high, reflecting their low availability in the environment, possibly due to the small founder population size with a limited number of North American parasites [52]. In addition to high environmental tolerance [21], low parasite load may thus be another trait of non-native pumpkinseed populations in Türkiye associated with rapid adaptation to new environmental conditions and establishment success, as was the case with pumpkinseed in the Sarıçay River [80].

Investigation of parasites in pumpkinseed in Türkiye has mainly focused on the European part of the country [81,82], with a single study from Asia focused on the rosette agent *Sphaerothecum destruens* Arkush, Mendoza, Adkison and Hedrick, 2003 in the Sarıçay River [83]. The previous studies [81,82] reported only 11 parasite taxa in pumpkinseed from Lake Sığircı and Lake Gala, both located in the Meriç River basin. These taxa included monogeneans *Gyrodactylus* spp. and *Onchocleidus similis* (Mueller, 1936), metacercariae of *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899, *Diplostomum* sp., *Tetracotyle* sp., *Tylodelphys clavata* (von Nordmann, 1832) Diesing, 1850, acanthocephalan *Acanthocephalus* sp., nematodes *Eustrongylides excisus* Jägerskiöld, 1909 and Nematoda gen. sp., copepod *L. cyprinaceae*, and unidentified glochidia (summarised by [84]). Out of the parasite taxa previously found on pumpkinseed in Türkiye, just a single species, crustacean *L. cyprinaceae*, was also found in this study. Our study identified at least seven additional parasite species infecting pumpkinseed in Türkiye, increasing the number of known parasite taxa associated with this fish species in the country. This finding suggests a richer parasite fauna in pumpkinseed populations than previously documented.

The co-introduction of parasites to new areas along with their hosts may be limited due to bottlenecks resulting from small founder populations [85]. Additionally, the probability of parasite co-introduction may depend on factors such as the host's origin (from low or high parasitised sources) or developmental stage (e.g., eggs, larvae, vs. adults; [52]). Our study confirms the presence of three North American parasite species in Türkiye: the

myxozoan *M. dechtiari*, the monogenean *O. dispar*, and the digenean *P. centrarchi*. These species were previously found in other European pumpkinseed populations (e.g., [38,45]). Notably, this study represents the first record of these North American parasite species in Türkiye; two (*O. dispar* and *P. centrarchi*) were observed in the Asian part of the country, while all three species were found in the European part. Interestingly, our study did not detect *O. similis*, previously found by Çolak [81] and Soylu [82] in lakes in the western part of the country. Dactylogyrid monogeneans are often host specific [86], and both *O. similis* and *O. dispar* primarily infect centrarchid fish [87]. The presence of different *Onchocleidus* species in both previous and current studies suggests potentially different sources of pumpkinseed for the localities in Gala and Sığircı Lakes [81,82] and our sampling localities.

Onchocleidus dispar represents the most widespread and well-established co-introduced parasite in European pumpkinseed populations among the three species mentioned. Its presence in Europe dates back to the 1950s [88], making it one of the longest-known co-introduced parasites in pumpkinseed. Among the eight American monogeneans co-introduced to Europe [46,47], *O. dispar* represents the majority of single-species infections in many distant European populations. Records of single-species monogenean infection of pumpkinseed by *O. dispar* have been reported in various European regions, including southwest England [44], the basins of the Oder in Poland [36], the Dnieper in Ukraine [89,90], the Danube in the Czech Republic [38], and, in fact, the reservoirs on the Sado and Tejo rivers in Portugal [38]. Despite the high microsatellite similarity observed between pumpkinseed populations in Türkiye, indicating a common source of introduction, *O. dispar* was not observed in the Aegean region of Anatolia, specifically in the Sarıçay River and Dipsiz-Çine Stream. The absence of this parasite, which is specific for pumpkinseed and does not require other hosts in its life cycle, suggests either unfavourable environmental conditions for the parasite during or after pumpkinseed translocation or the introduction of very young or treated fish.

Posthodiplostomum centrarchi has recently been reported from various countries in Europe infecting both pumpkinseed and largemouth bass *Micropterus salmoides* (Lacepède, 1802) (e.g., [36,38,50,51,91]), being distributed by its definitive hosts, herons (Ardeidae) [51]. Parasites collected in Sarıçay River and Değirmenköy Reservoir genetically corresponded to specimens obtained in Hungary and Portugal [50,92], as well as *Posthodiplostomum* sp. 3 sensu Locke et al., 2010, recovered from pumpkinseed in North America [93]. This study expands the distributional range of this non-native parasite to the Asian continent. Among the three North American parasites found, myxozoan *M. dechtiari* is the most recently observed, with only two reports from Europe. It was first observed in Hungary by Goswami et al. [45], and later, Kvach et al. [89] found it in the basin of the lower reach of the Danube River in Ukraine. This study marks the third report of this parasite in Europe.

The pumpkinseed in Türkiye harboured six parasite taxa, including one crustacean species, *L. cyprinacea*, and five taxa of larval nematodes. Most of these species occurred only occasionally, except for two relatively prevalent nematodes. *Paraquimperia tenerrima*, recorded solely in the Sarıçay River, was the most abundant parasite species, with a mean abundance of 6.6. In contrast, the mean abundance of other acquired parasites did not exceed one (see Table 4). This finding is also the first report of this parasite species in pumpkinseed. *Paraquimperia tenerrima* is a stenoxenous parasite known to infect the European eel (*Anguilla anguilla* L., 1758) as the definitive host, and it uses various fish species as paratenic hosts [94]. It is an indicator parasite of the eel populations, found mainly in old-age fish individuals [95]. Pumpkinseed, apparently susceptible paratenic hosts, had nearly 50% of individuals infected, with a maximum intensity of 70 larvae per fish. Further translocation of pumpkinseed infected with this parasite may spread *P. tenerrima* to other localities and subsequently infect local eel populations.

The second nematode parasite found in pumpkinseed with high prevalence (57%) were larvae of *Eustrongylides* sp. Despite primarily infecting fish-eating birds, larvae of *Eustrongylides* have zoonotic potential, being agents of human diseases [94]. Although rare, representatives of *Eustrongylides* have caused eustrongylidosis in humans in North

America, the Middle East, and Africa [96], potentially having socio-economic implications. Therefore, the continued expansion of non-native pumpkinseed populations in Türkiye poses a threat not only to natural conservation efforts and fisheries but also potentially to human health.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author, [M.O.], upon reasonable request.

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