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Genetic differentiation between captive and wild populations induced by selective breeding in morphologically diverse *Takifugu* pufferfish

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Pufferfish from the genus *Takifugu* are vital commercial resources in East Asia. Within the genus, the taxonomic status of two commercially important species, *T. rubripes* and *T. chinensis*, remains ambiguous, especially given their morphological variability. Recent observations of suspected hybrids between *T. rubripes* and *T. chinensis* on Jeju Island, South Korea, displaying intermediate phenotypes, have further confused their classification. In this study, we analyzed 73 pufferfish, including wild-caught *T. rubripes*, *T. chinensis*, suspected hybrids, and farm-bred *T. rubripes*, using 16 microsatellite loci to explore their population structure and evolutionary relationships. The Bayesian clustering and principal coordinate analysis showed minimal genetic differentiation among the wild populations, regardless of phenotype. This finding suggests that *T. rubripes* and *T. chinensis* might represent a single species with considerable morphological diversity. In contrast, farm-bred *T. rubripes* exhibited significant genetic differentiation from wild populations, likely due to domestication-induced genetic drift. These results challenge the existing taxonomic distinctions between *T. rubripes* and *T. chinensis* and highlight the profound impact of aquaculture on the genetics of captive populations. This study underscores the necessity for ongoing research into the taxonomy and population genetics of the *T. rubripes-chinensis* complex to guide conservation and management strategies and stresses the importance of genetic monitoring in pufferfish aquaculture to counteract inbreeding and genetic drift.

KEYWORDS

***Takifugu*, microsatellites, population structure, genetic drift, domestication**

1 Introduction

Pufferfish species of the genus *Takifugu* are widely consumed as a culinary delicacy in South Korea, Japan, and China, holding a significant position in the gastronomic culture of these countries. In particular, *Takifugu rubripes* is highly esteemed for its exquisite texture and flavor, making it a species of considerable economic value and a widely cultivated target in aquaculture. As of 2022, approximately 16,000 tons of farmed *T. rubripes* were produced in China and around 3,000 tons in Japan (FAO, 2024). The global aquaculture production of this species is on the rise, reflecting the increasing market demand for pufferfish. However, the aquaculture production of *T. rubripes* in South Korea remains limited, with annual production of less than 70 tons, primarily confined to specific regions such as the southern part of Jeju Island (FAO, 2024). Consequently, the majority of pufferfish consumed domestically relies on annual wild catches of 3,000 to 5,000 tons, supplemented by imports of approximately 3,000 to 5,000 tons of pufferfish from China.

Pufferfish contain tetrodotoxin (TTX), a potent neurotoxin, and TTX-related food poisoning incidents have primarily resulted from improper consumption of pufferfish (Guardone et al., 2020). Such incidents have been consistently reported in countries where pufferfish are consumed (Kim et al., 2003; Watari et al., 2021). The distribution of this toxin within tissues varies among different pufferfish species (Noguchi et al., 2006b; Noguchi and Arakawa, 2008); therefore, accurate species identification is essential for preventing TTX poisoning by precisely determining the toxin distribution, and consumption is restricted to specific pufferfish species with verified edible parts by the Ministry of Food and Drug Safety of Korea. While the accumulation of TTX in wild pufferfish poses a threat to food safety, farmed pufferfish, which can be systematically managed, may serve as a viable alternative for the safe supply of seafood. Since pufferfish acquire TTX through exogenous sources, primarily from their diet, they do not accumulate TTX in their bodies when reared in environments isolated from TTX sources, such as aquaculture facilities (Noguchi et al., 2006a). Therefore, continuous management of the pufferfish aquaculture industry is essential to ensure the supply of pufferfish as a safe seafood product free from the risk of TTX poisoning. However, despite the commercial value of farmed pufferfish, the genetic impacts of selective breeding on farmed populations remain understudied. Assessing genetic diversity in both wild and farmed pufferfish is crucial to identify potential issues affecting aquaculture sustainability and productivity. Population structure is influenced by genetic drift and natural selection. It typically develops over generations but can form rapidly through founder effects or genetic bottlenecks (Peacock et al., 2009). In aquaculture, genetic drift is inevitable due to selective breeding, yet reports on genetic divergence from wild populations are rare (Barría et al., 2023). Severe genetic drift is well-documented in livestock but less reported in fish. However, population structure is evident in ornamental fish such as goldfish (Chen et al., 2020) and Betta fish (Zhang et al., 2022), which show significant genetic divergence from their wild counterparts due to domestication.

Two pufferfish species, *T. rubripes* and its closely related species *T. chinensis*, which are distributed in the coastal waters of South Korea, are morphologically distinguishable: *T. rubripes* features a white anal fin and irregular black spots on dorsal half of posterior part of the body, whereas *T. chinensis* does not exhibit these markings (Baek et al., 2018). Additionally, *T. rubripes* is known to spawn at depths of 20–50 m, whereas *T. chinensis* spawns at depths below 100 m, indicating distinct ecological differences between the two species. Furthermore, *T. rubripes* is generally recognized as a larger species compared to *T. chinensis* in terms of maximum body size (Matsuura, 2017). However, advances in molecular phylogenetics have prompted a reevaluation of the taxonomic classification within the genus *Takifugu* over the past several decades. The research consistently supports the notion that *T. rubripes* and *T. chinensis*, previously considered distinct species based on morphological differences, may be a single species. This hypothesis is backed by studies utilizing mitochondrial haplotypes, microsatellites, and genome-wide SNPs (Reza et al., 2011; Baek et al., 2018; Park et al., 2020; Takahashi et al., 2023; Liu et al., 2024). In addition, a noticeable increase in pufferfish displaying intermediate morphological traits between *T. rubripes* and *T. chinensis* has been observed in Korea, suggesting possible natural hybridization between the two species. Intermediate forms of this combination have also been observed in Japan (Reza et al., 2008), and since the edible parts (muscle, skin, and testes) of both species are identical, consumption is allowed only for pufferfish exhibiting these intermediate traits among hybrid pufferfish (Ministry of Health, Labour and Welfare of Japan, 2020). Despite the increasing occurrence of intermediate forms, information on their occurrence and genetic population structure remains insufficient. These potential hybrids, referred to here as "hybrid-suspected pufferfish" (HSP), may spark debate over the accuracy of existing species classifications and the broader ecological and evolutionary implications of interspecific hybridization. To address these issues, it is critical to explore the genetic population structure and evolutionary relationships between *T. rubripes*, *T. chinensis*, and the HSP. Such studies are crucial for clarifying taxonomic ambiguities and devising appropriate conservation and management strategies. Furthermore, examining the genetic underpinnings of observed morphological variations within this group could offer deeper insights into the drivers of phenotypic variation and adaptation in marine fishes.

While genomic techniques are invaluable for identifying signs of domestication and pinpointing genes linked to local adaptation, more accessible genetic markers, such as microsatellites, remain crucial. These markers are effective in deciphering population structures, detecting genetic drift impacts, and assessing inbreeding within domesticated populations, especially when they show polymorphism (Prunier et al., 2023). In this study, we utilized a suite of 16 microsatellite markers to examine the genetic relationships among wild-caught *T. rubripes*, *T. chinensis*, and HSP from the waters around Jeju Island, alongside farm-bred *T. rubripes* from local aquaculture operations. Our analysis of genetic diversity and population structure aimed to (1) clarify the taxonomic distinctions between *T. rubripes* and *T. chinensis*,

(2) provide genetic evidence of natural hybridization between these species, and (3) assess the genetic impacts of selective breeding on farmed *T. rubripes* populations. Our findings offer fresh insights into the evolutionary history and current genetic landscape of the *T. rubripes-chinensis* species complex, which hold significant implications for their taxonomy, conservation, and sustainable management.

2 Materials and methods

2.1 Sampling and DNA extraction

From 2020 to 2022, a total of 73 pufferfishes were purchased from the market on the east, south and Jeju Island and an aquafarm in Jeju Island (Figure 1, Table 1). Based on phenotypic features, obtained pufferfishes were identified as 4 groups; *T. rubripes*, *T. chinensis*, and HSP type 1 and 2. *T. rubripes* with white anal fin and irregular black marks on the side of the body can be distinguished by *T. chinensis* (Figures 1, 2, Supplementary Table S1). HSP have phenotypic features of both species. HSP type1 are characterized by a white anal fin (*T. rubripes*) and no irregular black marks on the side of the body (*T. chinensis*), and the HSP type2 have a black anal fin (*T. chinensis*) and irregular black marks (*T. rubripes*, Figure 2, Supplementary Table S1). Genomic DNA used for microsatellite analysis was extracted from the pectoral fin using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols.

2.2 Molecular identification

For the molecular identification of the species, we performed DNA barcoding using the primer that targeted the mitochondrial COI gene (Fish F1: 5' TCA ACC AAC CAC AAA GAC ATT GGC AC 3' and R1: 5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3' (Ward et al., 2005). We employed primers commonly used in teleosts for the DNA barcoding analysis and followed the PCR conditions described by (Ward et al., 2005). The PCR products were verified through 1% agarose gel electrophoresis, and the positive products were submitted to Macrogen for sequencing. The obtained sequencing results were used to identify the species based on the most similar species to the query using BLAST. Maximum likelihood tree was reconstructed to address the phylogenetic position among closely related species using the software IQ-TREE2 with default parameters (-b 1000) (Minh et al., 2020).

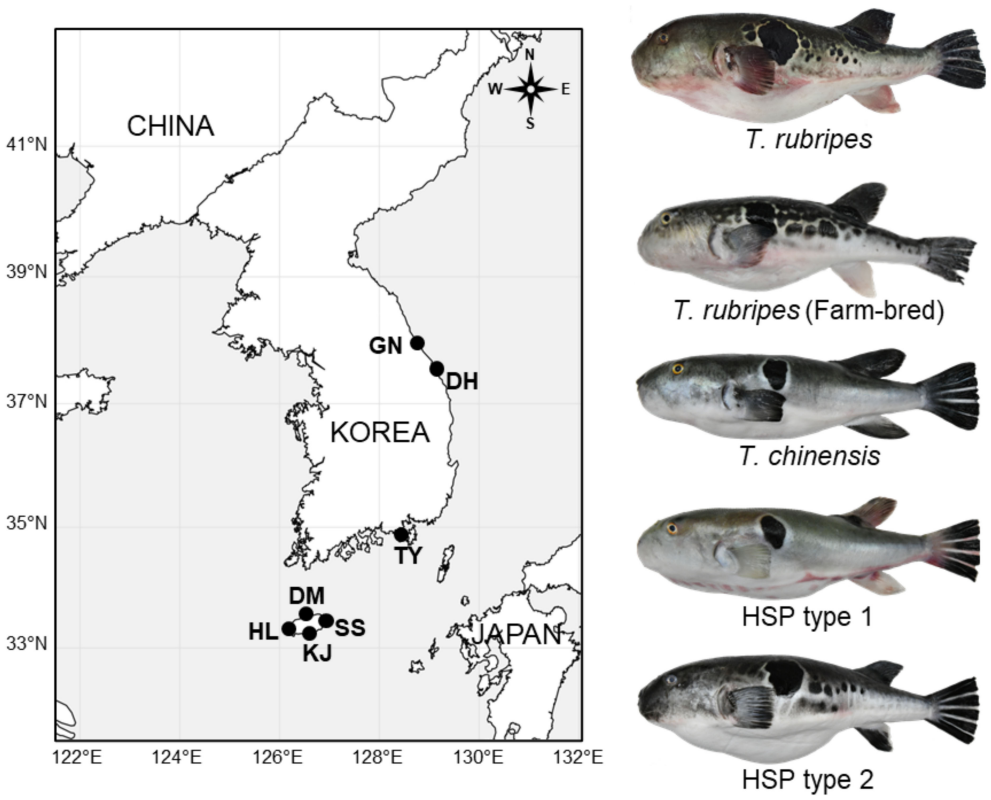

FIGURE 1
Sampling locations of *Takifugu rubripes* and *T. chiensis* in South Korea. *T. rubripes* (wild type), *T. rubripes* from fish farm, *T. chinensis*, and two types of hybrid suspect pufferfish (HSP) are shown with representative images. Other abbreviations on the map refer to locality of South Korea where these fish species have been collected: HL (Hallim market, Jeju), KJ (Kangjeong market, Jeju), DM (Dongmun market, Jeju), SS (Seongsan farm, Jeju), TY (Seoho market and offshore collection, Tongyeong), DH (Mukhwo market, Donghae), and GN (Jumunjin market, Gangneung).

TABLE 1 List of *Takifugu rubripes-chinensis* complex samples collected.

Species	Locality	Sampling Date	Sample size	Status
T. rubripes	Gangneung	2020-12-29	1	Wild
	Jeju	2020-12-08	1	Wild
		2020-12-24	1	Wild
	Tongyeong	2020-10-22	1	Wild
		2022-02-06	6	Wild
T. rubripes	Jeju	2022-02-21	19	Farm-bred
T. chinensis	Jeju	2020-11-25	4	Wild
		2022-03-10	19	Wild
	Seogwipo	2022-02-24	1	Wild
	Tongyeong	2022-02-06	4	Wild
HSP type 1	Jeju	2022-03-10	2	Wild
HSP type 2	Donghae	2020-03-16	3	Wild
	Jeju	2020-10-15	4	Wild
		2020-03-08	3	Wild
	Tongyeong	2020-10-22	4	Wild

2.3 Microsatellite analysis

The amplification efficiency and the polymorphism for some of the samples were examined in 31 primer sets developed from *T. rubripes* and *T. pseudommus* using electrophoresis, and a total of 16 primer sets were finally selected. We performed the polymerase chain reaction (PCR) to amplify selected microsatellite loci, and the information of each microsatellite marker were summarized in Table 2. Forward primers of selected 16 microsatellite markers were 5′-fluorescently labeled using 6-FAM, HEX, or TAMRA to distinguish different alleles on the sequence analyzer. The PCR was conducted in a total volume of 50 μL, containing 100 ng of template DNA, PCR Master Mix 2X (ThermoFisher), and 10 μM of each primer. For Cst-1 and Cst-4, The PCR conditions were as follows: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 58°C (Cst-1) or 60°C (Cst-4) for 1 min, extension at 72°C for 2 min, and final elongation at 72°C for 10 min. For the other markers, The conditions were as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C (Tru-7, Tru-8) or 60°C (Tru-9, Tru18, Fms13, F160, F112, F178, F86, F61, Tru-17, F65, F160, and F204) for 30 sec, extension at 72°C for 30 sec, and final elongation at 72°C for 1 min or 2 min. The amplification of fragments were confirmed by electrophoresis in 2.5% agarose gels. Fragment analysis of amplified products was performed by Macrogen Inc. (Seoul, Korea) using ABI 3730xl analyzer (Applied Biosystems, Foster City, CA, USA), and allele size was determined using the Peak Scanner Software v.1.0 (Applied Biosystems). The scored allele size data were evaluated for the existence of null alleles, allele dropout and stutter, and other scoring errors using MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004).

2.4 Genetic diversity and population structure analysis

The total number of alleles per locus (A), allelic richness (Ar), and observed and expected heterozygosity (H_O and H_E) were calculated using GenAlex v.6.51 (Peakall and Smouse, 2012) and FSTAT v.2.9.4 (Goudet, 2001). Arlequin v.3.5.2.2 was used to calculate the pairwise F_{ST} values between populations based on microsatellite loci with 1,000 permutations (Excoffier and Lischer, 2010). The genetic structure of populations was estimated using the Bayesian model-based clustering method implemented in the software STRUCTURE v.2.3.4 (Pritchard et al., 2000). The burn-in period was set to 5,000 and the number of Markov chain Monte Carlo (MCMC) repetitions to 50,000. Twenty iterations for each K-value were carried out assuming cluster K from 2 to 9 to examine population structure. The optimal number of genetic clusters was estimated using STRUCTURE harvester v.0.6.94 (Earl and vonHoldt, 2012), and CLUMPAK (Cluster Markov Packager Across K) was used to visualize the STRUCTURE output (Kopelman et al., 2015). Principal Coordinates Analysis (PCoA) based on tri distance matrix were used to visualize the genetic relationships among individuals and populations using covariance-standardized method as implemented in GenAlex v.6.51 (Peakall and Smouse, 2012). Contemporary effective population size was measured using NeEstimator to assess the effect of domestication on the bottleneck. Hybrid index for the putative hybrids between *T. rubripes* and *T. chiensis* was estimated using Genodive (Meirmans, 2020) with default parameters. We used the software POPTREE2 (Takezaki et al., 2014) to reconstruct the phylogenetic relationship of the five groups. Trees were generated using the neighbor-joining (NJ) method with 1,000 bootstrap replicates.

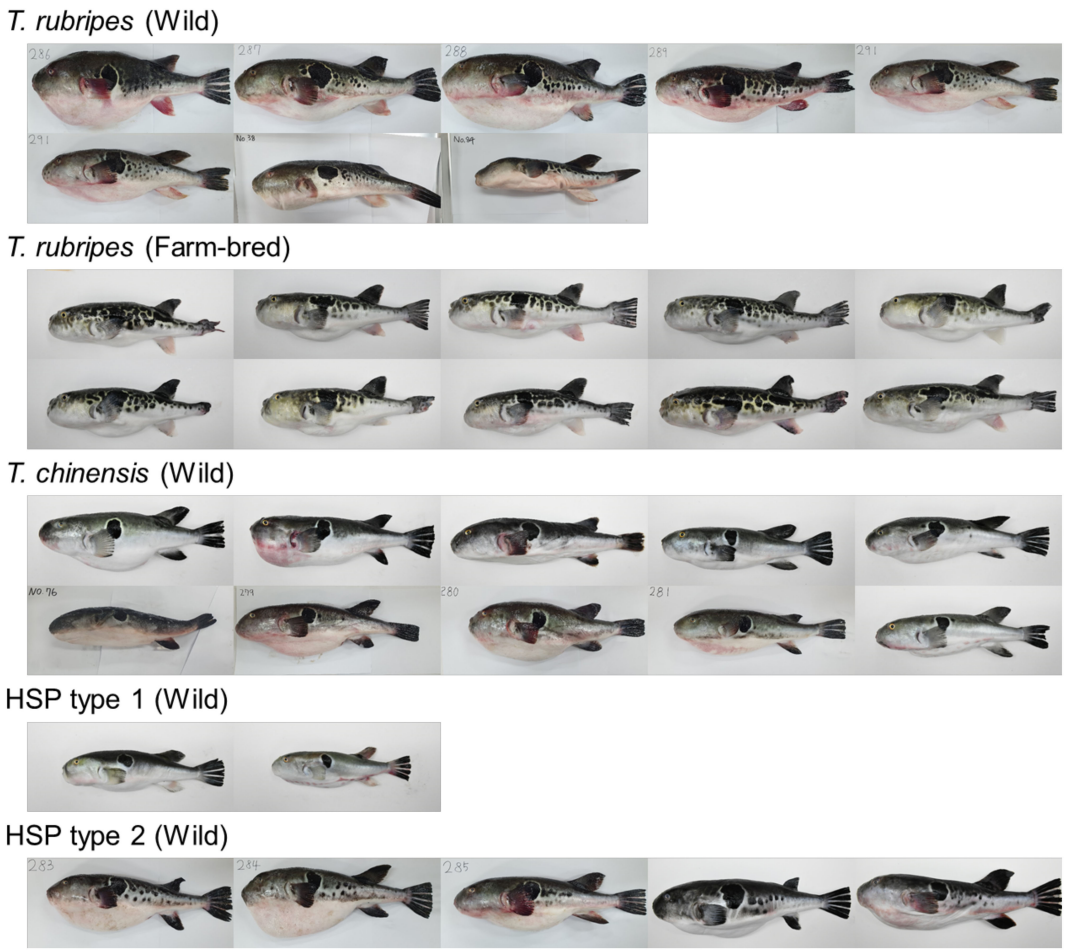

FIGURE 2
Lateral view of representative individuals used in this study.

3 Results

3.1 Species identification

The COI barcode sequences obtained in this study showed 100% similarity between *T. rubripes* and *T. chinensis*, making it impossible to distinguish between the two species using the barcode sequence. All samples used in this study matched the sequence of *T. rubripes-chinensis* species complex. The ML phylogeny showed that the individuals used in this study clustered within the *T. rubripes-chinensis-pseudommus* complex (Supplementary Figure S1). No significant differences in the color of the anal fin were observed between wild and farmed *T. rubripes* (Figures 1, 2), while an inter-specific level difference was observed between *T. rubripes* and *T. chinensis*. A substantial difference in the distribution of black spots on the body was observed between the wild and farmed populations of *T. rubripes*. The farmed individuals showed larger spots on the body, while the wild population showed smaller spots that were similar in size to the diameter of the eye. Two hybrid-suspected groups (HSP type 1 and 2) exhibited the mosaic pattern in their spots on the body and color of the anal fin.

3.2 Genetic diversity of pufferfish

Sixteen microsatellite markers were used to measure the genetic diversity of 73 individuals, including *T. rubripes*, *T. chinensis*, and HSP type 1 and 2. The results showed that the species-level genetic diversity varied in five groups. The number of alleles varies considerably, from 3.438 in HSP type 1 to 15.563 in *T. chinensis* (Table 3). Observed and expected heterozygosity were relatively consistent across populations, ranging from 0.724 to 0.875 for observed heterozygosity and 0.664 to 0.878 for expected heterozygosity. Overall, the descriptive index suggests the domesticated group exhibits lower genetic diversity than wild populations (Table 3). No significant differences were observed in HSP types. Hybrid index analysis revealed that two HSP type 1 individuals showed affinity with *T. chinensis* (h = 0.000 - 0.211, zero indicates affinity with *T. chinensis*). For the HSP type 2 individuals, hybrid indexes were varied in samples (h = 0.000 - 0.614). The estimated effective population size (N_e) for each population was infinite for all populations except for the farmed *T. rubripes* population (N_e = 10.9, 95% CI = 8.4 - 14.3).

TABLE 2 List of microsatellite markers used.

Locus	Repeat motif	Primer sequence (5′->3′)	Reference
Tru-7	$(AC)_{17}$	F: AAAGACAAAGACAAAAAGCAG R: TTGTCGTACACTTCAAGATTT	Takagi et al., 2003
Tru-8	$(AC)_{22}$	F: AATGTCTAGGATTTTCAGTGG R: CCAGCTCTGTCCCCCAATACA	Takagi et al., 2003
Tru-9	$(GT)_{21}$	F: CTCTCATCCTCCGAAAAGCAC R: ATCAGCCAAACCCCTCTAATA	Takagi et al., 2003
Tru-18	$(AC)_{25}$	F: CATCCTCATCAGGGTCACAGA R: TTACATAAGGGCCACAAAGTT	Takagi et al., 2003
Cst-1	$(AC)_{17}$	F: CCTTTTCCTTAGTTTAGCCA R: GCCTGTATTTCCATTTGACTG	Cui et al., 2005
Cst-4	$(AAC)_{8}$	F: TCTGCTGGGATGAACGCCTAA R: GCCGTTTCTGACCACAATCC	Cui et al., 2005
Fms13	$(TGTA)_{13}$	F: TCCTCCATTATCCCAGTTGGT R: GGATCATTATAAGATGGTGC	Furukawa et al., 2004
F112	$(CA)_{39}$	F: GACAGCACGAGTCAGTTTCG R: AAAGATGGCTGCTGTGTCG	Kai et al., 2005
F178	$(CA)_{32}$	F: TCGTGTGTCCCCATTCTACA R: TGTTGGCAATGACGTGACTC	Kai et al., 2005
F61	$(CA)_{25}$	F: TCAACCCAGAACACCACAAA R: TACCCCGAATTAGTGCAAGC	Kai et al., 2005
F86	$(CA)_{21}$	F: CCAAAGCCTGTTCAAACACC R: CATGGACCGTGACCACATAG	Kai et al., 2005
Tru-17	$(GCCA)_{18}$	F: ATGCATAGGATGAAGGACACT R: TGGTTAGCTGCAGGGTTTTAG	Takagi et al., 2001
F65	$(CA)_{63}$	F: TCAAATCAAGCGCAGACAAC R: GTTGCAACATTGTGGACTCG	Kai et al., 2005
F160	$(CA)_{49}$	F: GCAGTGTGGGAAGGGATAAA R: TTTACCGCCCTTTGTTTCAC	Kai et al., 2005
F204	$(CA)_{50}$	F: GCACTGGTGCTCACAGAAGA R: CAGAAGGGTCCATCACCACT	Kai et al., 2005
F153	$(CA)_{43}$	F: CAGCGCACTGAACTCACCTA R: CCTGGAGCATCGTCACATTA	Kai et al., 2005

3.3 Intraspecific population structure

The highest F_{ST} is observed between farm-bred *T. rubripes* and others, ranging from 0.134 to 0.158, while other comparisons were below 0.03 (Table 4). The wild *T. rubripes* and *T. chinensis* exhibit no significant differentiations ($F_{ST} = 0.022$, $p < 0.05$). According to Frankham et al. (2002), F_{ST} value greater than 0.15 can be considered significant in differentiating populations. Our STRUCTURE analysis indicated that the significant population structure was observed between farm *T. rubripes* and the others (Best $K = 2$), while no significant structure was observed among wild *T. rubripes*, wild *T. chinensis*, and the moderate groups (HSP1 and HSP2) (Figure 3A). The PCoA plot revealed the distinct clustering patterns, with two wild pufferfish populations and the moderate groups forming a cluster together, while *T. rubripes* from the farm showed a distinct cluster from others (Figure 3B). The first two principal coordinates account for 12.18% and 3.85% of the total genetic variation, respectively. Interestingly, farmed *T. rubripes* shows the genetic distinctiveness within the group. The genetic distance-based tree indicated that farmed *T. rubripes* showed the sister relationship with wild *T. rubripes* although moderate bootstrap support value (Supplementary Figure S2).

4 Discussion

In this study, we performed the genetic analysis using 16 microsatellite markers to determine the population structure and evolutionary relationships of *T. rubripes* and *T. chinensis* throughout Korean waters. Our results revealed no significant population structure throughout the individuals except for the farmed *T. rubripes* population. No clear genetic differentiation was observed in the two species, which was consistent with the previous studies using mitochondrial markers to genomic SNP markers (Baek et al., 2018;

TABLE 3 Comparisons of genetic diversity index among five groups of *T. rubripes-chinensis* complex.

Population	*N*	*A*	A_R	H_O	H_E
T. rubripes (Wild)	10	11.188	3.491	0.875	0.856
T. rubripes (Farm-bred)	19	5.188	2.544	0.724	0.667
T. chinensis	28	15.563	3.446	0.844	0.878
HSP type 1	2	3.438	3.438	0.844	0.664
HSP type 2	14	12.250	3.485	0.817	0.873

N, number of individuals; *A*, number of alleles; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity.

Park et al., 2020; Takahashi et al., 2023; Liu et al., 2024). The individuals with moderate morphological characteristics between species were also indistinguishable in terms of allele frequency. However, the estimated genetic diversity for the HSP group should be interpreted with caution due to the limited sample size. This implies that the species identity of *T. rubripes* and *T. chinensis* should be reconsidered given the fact that no genetic and genomic divergences between the species (Reza et al., 2011; Baek et al., 2018; Park et al., 2020; Takahashi et al., 2023). The fact that the species complex possesses diverse phenotypic characteristics despite having no genetic divergence suggests that the phenotypic traits (i.e., the coloration of the anal fin and lateral bands on the body) of this species complex exhibit intraspecific morphological variation. Our study highlights that the severe genetic drift during domestication is responsible for the rapid change of allele frequency, which may have resulted in the population structure.

Due to its economic significance, species identification of *Takifugu* species using genetic and genomic markers has been studied for decades (Reza et al., 2011; Baek et al., 2018; Park et al., 2020; Takahashi et al., 2023; Liu et al., 2024). Yet, there was no evidence to support the genetic isolation between *T. rubripes* and *T. chinensis*. We used the microsatellite markers developed by five independent research groups (Takagi et al., 2001, 2003; Furukawa et al., 2004; Cui et al., 2005; Kai et al., 2005) and found no differences in allele frequencies between the two species. Given that different species become reproductively isolated, thereby limiting gene flow, and consequently possess different allele frequencies across their genomes, this can be detected through allele frequency among the randomly selected markers. Previous studies that investigated the differences in allele frequencies of SNPs in genomic regions also indicated that the lack of clear differences in allele frequencies suggests that these two species are genetically undifferentiated and belong to the same species. Furthermore, the sharing of mitochondrial haplotypes, considering the characteristics of mitochondrial loci reflecting historical differentiation, suggests the possibility that the two species have not historically differentiated and may be the same species. Indeed, our result of the hybrid index clearly indicate that no signal of gene flow between species due to the lack of genetic divergence between the species. The distinct morphological characteristics observed in pufferfish may be attributed to the influence of environmental variables, including water temperature and salinity, on their developmental processes. Further investigation is necessary to ascertain the extent to which environmental factors shape morphological diversity.

Pufferfish have adapted to various aquatic ecosystems (Yamanoue et al., 2009; Santini et al., 2013). Understanding the functional and evolutionary significance of their diverse phenotypic characteristics can contribute to the effective management and conservation of wild populations. By exploring the adaptive mechanisms that have enabled pufferfish to thrive in different environments, we can gain valuable insights into their ecological roles and develop strategies to protect their habitats and maintain their genetic diversity. Furthermore, the study of phenotypic variation in pufferfish can shed light on the underlying molecular and developmental processes that give rise to phenotypic variation. This knowledge can be applied to other species facing similar environmental challenges, advancing our understanding of how organisms respond to changing conditions and adapt to new niches. Consequently, recognizing the significance of phenotypic variation in pufferfish and investigating its evolutionary and functional implications are crucial for effective conservation and management.

The effects of strong natural selection and genetic drift resulting from reduced effective population size and long-term inbreeding are the most prominent features of domestication (Nei and Tajima, 1981). This study observed a marked differentiation in allele frequencies in the cultured population. PCA and STRUCTURE

TABLE 4 Pairwise F_{ST} between five groups of *Takifugu rubripes-chinensis* complex.

	T. rubripes (Wild)	*T. rubripes* (Farm-bred)	*T. chinensis*	HSP type 1
T. rubripes (Farm-bred)	0.134			
T. chinensis	0.022	0.143		
HSP type 1	0.000	0.000	0.000	
HSP type 2	0.013	0.158	0.007	0.000

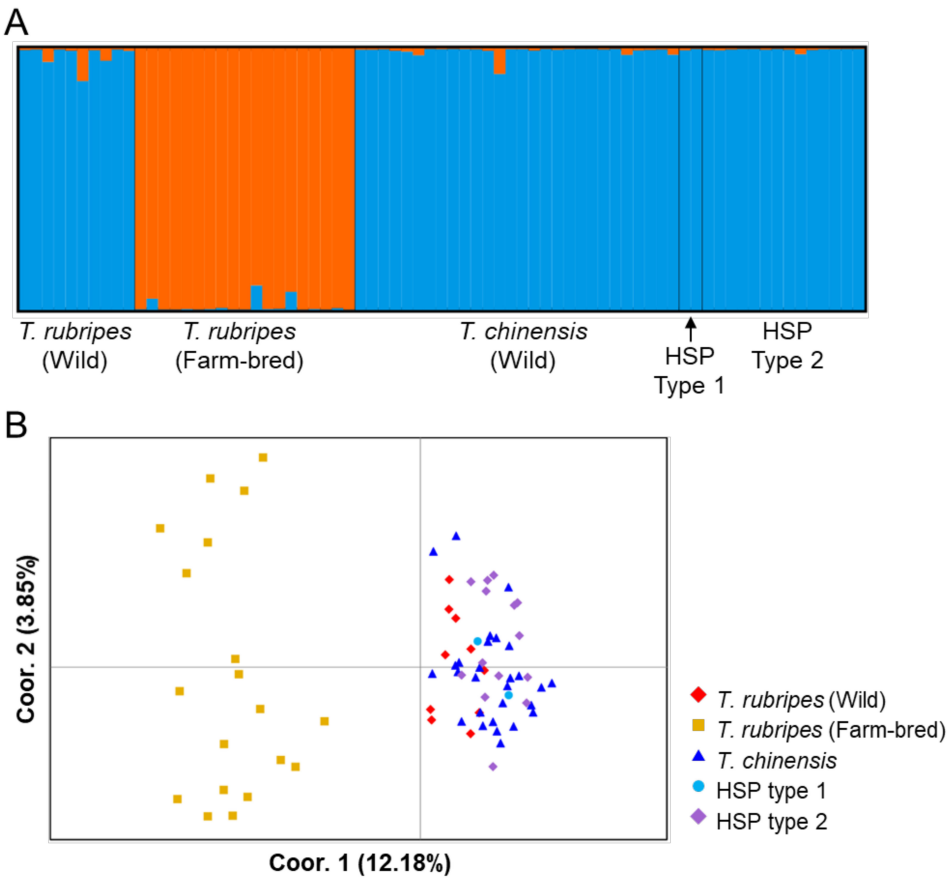

FIGURE 3
STRUCTURE plot (Best *K* = 2) of *Takifugu rubripes-chinensis* complex showing the proportion of genetic clusters (Q-values) on the Y-axis **(A)**. PCoA plot of the *T. rubripes-chinensis* complex **(B)**.

also strongly supported the differentiation between the cultured population and the wild *T. rubripes-chinensis* complex. Furthermore, extremely low effective population size ($N_e \approx 10$) and heterozygosity indicate reduced genetic diversity. When the effective population size is lower than 50, it indicates that the population is at a high risk of genetic drift and inbreeding (Jamieson and Allendorf, 2012; Ralls et al., 2018).

The changes in allele frequencies across 16 unlinked loci distributed throughout the genome could be attributed to strong selection and genetic drift. Although our study could not determine the specific types of selective pressures acting on these loci, the absence of intentional selection on parental individuals in the fish farm suggests that the observed changes may be due to genetic drift. To further investigate whether selection-driven differentiation has contributed to the major genomic differentiation, future research should employ genome-wide deep sequencing data and GWAS (genomewide association study) to detect genomic regions displaying genetic differentiation between wild and cultured populations. By examining whether these regions are the primary contributors to the overall genomic differentiation, it will be possible to elucidate the role of selection-driven differentiation in promoting genome-wide differentiation, which in turn leads to phylogenetic divergence.

Decreased genetic diversity can lead to problems, such as inbreeding depression, increased mortality rates, and reduced productivity in the aquaculture industry (Ye et al., 2022). Inbreeding depression is a phenomenon where the offspring of genetically related individuals exhibit reduced fitness, which can manifest as lower survival rates, slower growth, and decreased resistance to diseases (Smallbone et al., 2016). In aquaculture, this can result in significant economic losses due to reduced yields and increased susceptibility to diseases (Doyle, 2016). Furthermore, the loss of genetic diversity can limit the ability of a population to adapt to changing environmental conditions, such as climate change or emerging pathogens. This lack of adaptability can threaten the long-term sustainability of the aquaculture industry (Regan et al., 2021; Li, 2022). Therefore, to ensure the sustainable maintenance and growth of the aquaculture industry, further research is required to investigate the negative effects of reduced genetic diversity and develop strategies to mitigate these impacts (Robinson et al., 2023). This may include implementing breeding programs (i.e., genetic rescue strategy) that prioritize the maintenance of genetic diversity, exploring the use of wild broodstock to introduce new genetic material, and developing tools for monitoring and managing genetic diversity in aquaculture populations (Li, 2022; Robinson et al., 2023).

In conclusion, our microsatellite analysis of the *T. rubripes-chinensis* species complex challenges the current taxonomic distinction between *T. rubripes* and *T. chinensis*. The lack of genetic differentiation among wild populations, despite the substantial morphological variation, suggests that these nominal species may represent a single species expressing high phenotypic variation. This finding has important implications for the management and conservation of wild *Takifugu* populations, as well as for the regulation of the pufferfish food trade, given the differences in toxicity between species. In stark contrast, the significant genetic divergence observed in farm-bred *T. rubripes* highlights the profound impact of aquaculture practices on the genetic makeup of captive populations. The rapid genetic drift and potential inbreeding associated with selective breeding underscore the need for genetic monitoring in pufferfish aquaculture to maintain the health and productivity of farmed stocks. Future research should focus on resolving the taxonomic status of the *T. rubripes-chinensis* complex using high-resolution genomic markers and investigating the genetic basis of toxicity variation within this group. Furthermore, the development of sustainable breeding programs that prioritize the maintenance of genetic diversity is essential to ensure the long-term viability of the *Takifugu* aquaculture industry. Our study emphasizes the importance of integrating genetic data into the management of both wild and captive populations to promote the conservation and sustainable exploitation of these economically and culturally significant pufferfishes. Given the lack of genetic differentiation between what were previously considered distinct species, conservation efforts should focus on preserving the overall genetic diversity of this species complex rather than managing them as separate taxa. Conservation strategies should include establishing genetic monitoring protocols, creating a management plan that minimize inbreeding, and potentially implementing genetic rescue approaches by periodically introducing wild genetic material. These conservation measures would not only help preserve this economically important species complex but also maintain its ecological functions in marine ecosystems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because We analyzed commercially available fishes.

Author contributions

NK: Formal analysis, Investigation, Writing – original draft. HK: Investigation, Writing – review & editing. HH: Investigation, Writing – review & editing. WL: Writing – review & editing. HT: Writing – review & editing. KC: Writing – review & editing, Funding acquisition, Supervision. HJ: Conceptualization, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2025.1437097/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Maximum likelihood tree of *Takifugu* species based on mitochondrial COI sequences. Bootstrap values shown at branch nodes. Accession numbers and species name are provided for each terminal branch.

SUPPLEMENTARY FIGURE 2
PopTree plot showing the relationship among five groups. The numbers in the node represent the bootstrap values.

SUPPLEMENTARY TABLE 1
Morphological characteristics of two *Takifugu* species and HSP types.

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