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Population Structure Using Mitochondrial DNA for the Conservation of *Liobagrus geumgangensis* (Siluriformes: Amblycipitidae), an Endemic Freshwater Fish in Korea

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Abstract: *Liobagrus geumgangensis* is a novel Korean fish species endemic to the Geumgang and Mangyeonggang River basins on the Korean Peninsula. During a survey of *L. geumgangensis*, the discovery of *Liobagrus mediadiposalis* as a potential threat prompted an investigation into *L. geumgangensis* genetic diversity and structure. Three populations of *L. geumagangensis* and one population of *L. mediadiposalis* were investigated using a 1024-bp sequence in the *cytb* region of mitochondrial DNA. The Mangyeonggang River of *L. geumagangensis* displayed the lowest haplotype diversity (H_d) within a range of 0.000–0.337, with one to two haplotypes (*h*). The Jecheon region of the Geumgang River for *L. geumagangensis* population had the highest nucleotide diversity (π) and was within the range of 0.00000–0.00066. The *h* of *L. mediadiposalis* population was 3, the range of H_d was 0.292, and π was 0.00231. Tajima's D (*D*) and Fu's Fs (*F*) were negative and non-significant in the LgGJ population. The genetic structure of *L. geumgangensis* had no shared haplotypes among the three populations. The discovery of *L. mediadiposalis* in the Geumgang River suggests the necessity of non-habitat conservation and population management of fish farms to conserve *L. geumgangensis*.

Keywords: genetic structure; genetic diversity; *Liobagrus geumgangensis*; *Liobagrus mediadiposalis*; catfish

Key Contribution: In this study, we investigated the genetic structure of *Liobagrus geumgangensis* in the Geumgang and Mangyeonggang River basins. Overall, three populations were investigated. Through findings on genetic diversity and genetic structure, we aimed to provide a comprehensive understanding of the measures necessary for the conservation of *L. geumgangensis*.

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1. Introduction

Liobagrus geumgangensis was identified as a new species in 2023 and is endemic to the freshwaters of the Geumgang and Mangyeonggang River basins on the Korean Peninsula [1]. *L. geumgangensis* and *L. mediadiposalis* are almost similar in appearance. However, *L. geumgangensis* and *L. mediadiposalis* show distinct morphological differences in pectoral-fin spines and rays. In addition, *L. geumgangensis* is recognized as a different species as it shows more than a 5.8% difference from *L. mediadiposalis* in the gene sequence using *cytb* [1]. Until 2023, *L. geumgangensis* was classified as a single taxon under the name *L. mediadiposalis*. However, a recent study in 2023 identified it as a new species, and information regarding its genetic structure and diversity before and after this classification remains undisclosed.

Korea's Geumgang River water system drains to the west, with structures such as the Daecheong Dam and weirs blocking the flow between the upstream and downstream

regions. In the case of the Mangyeonggang River water system, gene flow is blocked by many weirs. Thus, the construction of dikes and dams blocks genetic flow between upstream and downstream regions, making it difficult to identify differences in genetic structure. In the case of catfish species, it has been reported that after adapting to a habitat and then moving from that habitat to spawn or feed, they often return to the original habitat [2,3]. Therefore, the identification of conservation areas based on biased wrong information can result in enormous losses due to habitat destruction of the catfish family [2,3]. Among the catfish family, species that rarely migrate may have unique genotypes for each microhabitat [4]. This is presumed to be because the catfish family forms a unique territory. Therefore, detailed, rather than biased, information should be procured for newly discovered species for identifying conservation habitats.

Endemic species are generally overlooked and face a heightened risk of extinction [4,5]. Vulnerability is influenced by factors including the threat of human overexploitation, declining population size, low reproductive potential, habitat damage from human activities, and introduced species [6–8]. *L. geumgangensis* is experiencing population decline because of indiscriminate habitat destruction and lack of attention, potentially resulting in reduced reproduction. The introduction of species sharing similar ecological niches poses a threat to the fertility and persistence of endemic species in relatively confined habitats [9]. This lack of attention may lead to severe genetic contamination, resulting in mixed populations despite genetic differences across geographical discontinuities [5]. Understanding the genetic diversity and structure before such situations arise is crucial for the continuity and conservation of species [10,11].

The mitochondrial DNA (mtDNA) *cytb* gene has been widely used as a molecular marker to determine population structure and diversity in genetic studies of several taxa [12–16]. River ecosystems operate across multiple temporal and spatial scales, shaping the connectivity and population genetic structure of the species that inhabit these habitats [17]. To confirm the genetic structure of a river ecosystem, identifying differences in genetic structures between different populations is a fundamental requirement [18]. The genetic structure typically develops over generations owing to interruptions in genetic flow [19]. This diversity in genetic structure is advantageous for species conservation. Various genetic differences allow species to persist and help them adapt to environmental changes [9]. *Pseudopungtungia nigra* has the same habitat distribution as *L. geumgangensis* [19]. *P. nigra* shows significant genetic differences between the Geumgang and Mangyeonggang Rivers, dividing into two genetically distinct groups [19]. Therefore, it is expected that *L. geumgangensis* will also show significant genetic differences between the Geumgang and Mangyeonggang Rivers. Because *L. geumgangensis*, like *P. nigra*, has a distribution range limited to Geumgang and Mangyeonggang Rivers, it is considered important to identify differences in genetic structure.

Samples were collected to determine the genetic diversity and structure of *L. geumgangensis*. During the collection, a population of *L. mediadiposalis* (LmMJ) was unexpectedly discovered in the upper Geumgang River. Given the similar ecological niche between *L. mediadiposalis* and *L. geumgangensis* in the Geumgang River, the former poses a threat to the latter. Therefore, we investigated the genetic structure and diversity of *L. geumgangensis* using mitochondrial *cytb* and provided basic data for conservation. Additionally, we sought ways to conserve *L. geumgangensis* through a genetic investigation of the upper Geumgang River population of *L. mediadiposalis*.

2. Materials and Methods

2.1. Sampling and Genomic DNA Extraction

Sampling was conducted in August 2023, when the animals were adults. Three populations of *L. geumgangensis* and one population of *L. mediadiposalis* were sampled using fishing nets; detailed information is provided in Figure 1 and Table S1. A total of 79 specimens were sampled from four sites. It is challenging to distinguish *L. geumgangensis* and *L. mediadiposalis* using morphological characteristics. For the LmMJ population, NCBI showed

that LmMJ was 92% similar to *L. Geumgangensis*. Additionally, for the LmMJ population, NCBI confirmed that LmMJ was 99.71% similar to *L. mediadiposalis* (OP980987; Nakdong-gang River water system) through BLAST identity analysis. Therefore, we considered the LmMJ population to be *L. mediadiposalis*. *L. geumgangensis* and *L. mediadiposalis* are endemic to Korea, and research does not require approval from animal ethical committees. The pectoral fin tissues of the fish samples were preserved in 99% ethanol. Genomic DNA was extracted from preserved tissues of all specimens using a DNeasy Blood & Tissue kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions.

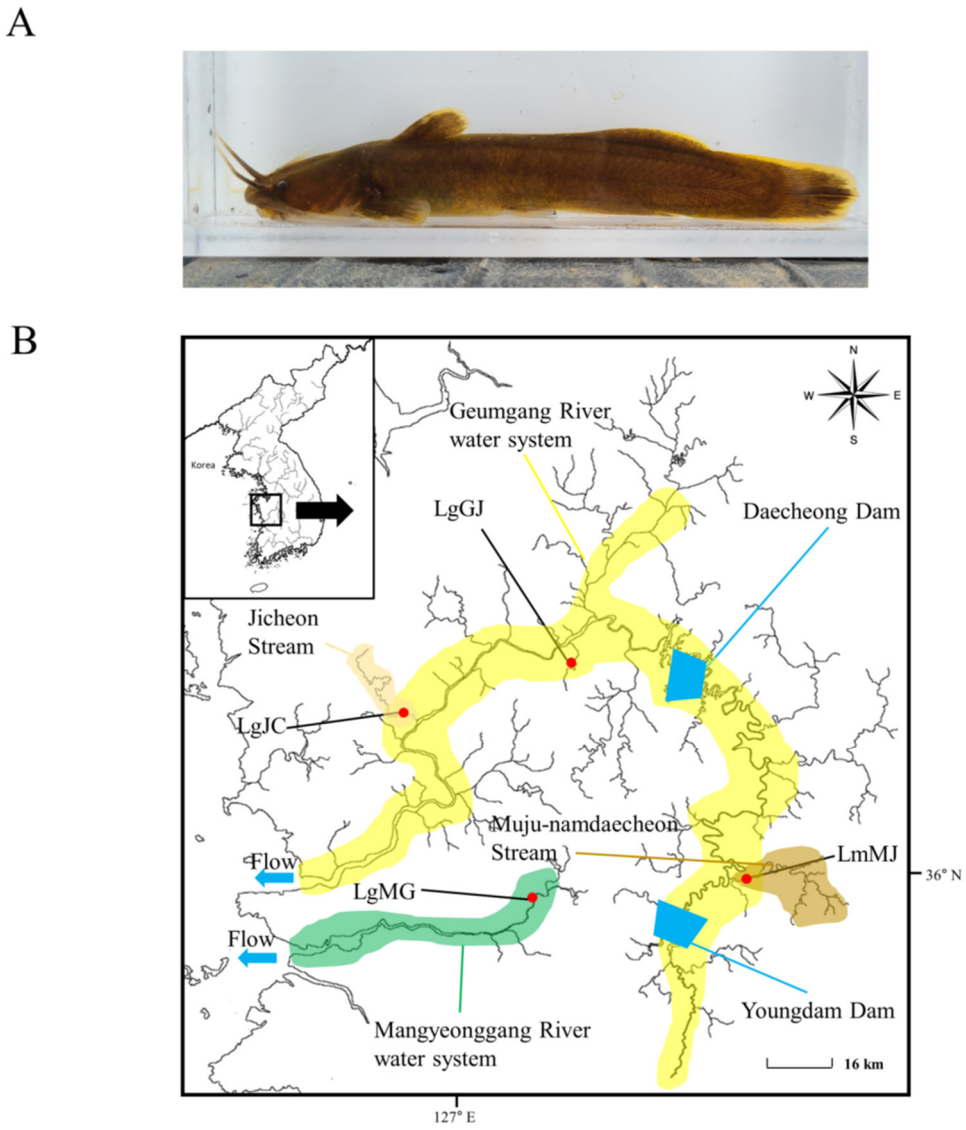

Figure 1. Specimen sample of *L. geumgangensis* (**A**). Sample location of four populations of *L. geumgangensis* and *L. mediadiposalis* (**B**). LgGJ, *L. geumgangensis* GJ population; LgJC, *L. geumgangensis* JC population; LgMG, *L. geumgangensis* MG population; LmMJ, *L. mediadiposalis* MJ population.

2.2. mtDNA Sequencing and Sequence Assembly

For mitochondrial gene selection, we selected a region of *cytb* with an appropriate level of haplotype diversity. Primers developed for *cytb* of mtDNA (Liobagrus_cytb_F1: TRAGAACTTATGGTAACCCGAA, Liobagrus_cytb_R1: GGATTACAAGACCGGCGCTT) were used in PCR, performed using a Mastercycler® pro gene amplifier (Eppendorf, Enfield, CT, USA). The AccuPower® PCR Premix Kit (BIONEER Co., Daejeon, Republic of Korea) was used for each reaction using 1 µL genomic DNA, 1 µL each of 1.0 µM forward and reverse primer, and 17 µL of tertiary distilled water. The PCR conditions were as follows:

pre-denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 57.5 °C for 30 s, and extension at 72 °C for 30 s, repeated for 30 cycles. The reaction concluded with a final extension at 72 °C for 10 min, followed by termination at 4 °C. The *cytb* PCR products were sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA). The *cytb* sequence thus determined was assembled using Geneious Prime 2022.2 (https://www.geneious.com, accessed on 11 November 2023) from raw data. The final determined *cytb* sequence is shown in the Fasta format in the Supplementary Materials. The *cytb* sequences were deposited in GenBank (PP061219–PP061226).

2.3. Sequence Analysis of Genetic Diversity and Structure in mtDNA

The alignment of *cytb* was conducted using the ClustalW algorithm within the MEGA software ver. 11.0.1 [20] based on *cytb* sequencing data. The number of haplotypes, haplotype diversity (H_d), nucleotide diversity (π), Fu's Fs (F), and Tajima's D (D) were calculated using DnaSP ver. 5.0 software [21]. A haplotype network was generated using Network ver. 10.2.0.0 [22] software, employing median-joining network analysis to determine the affinity between genotypes. STRUCTURE software (ver. 2.3.4) [23] was used to perform genetic structure clustering analysis based on the Bayesian model. We set the population constant (K) to 1–10 and applied a suitable admixture model to a mixture of water systems to estimate the most suitable population. Ten independent replicates with a burn-in of 10,000 iterations and Markov chain Monte Carlo (MCMC) with 100,000 iterations were performed. We analyzed a study by Evanno et al. [24] and the cluster results corresponding to K using STRUCTURE HARVESTER (ver. 0.7) [25] to estimate a population-appropriate constant (K). We used CLUMPK to estimate a graph of the appropriate K of the genetic structure of the population [26].

3. Results

3.1. Genetic Diversity

Cytb in mtDNA sets showed genetic diversity among the four populations (Table 1). The LgJC population showed the highest haplotype diversity (H_d) at 0.337. The π of *L. geumgangensis* populations ranged from 0.00000 to 0.00066, with the highest value being 0.00066 in LgJC and the lowest being 0.00000 in LgMG. For LgJC, F and D were positive, and for LgGJ, F and D were negative; however, these were not significant.

Table 1. *Cytb*-based genetic diversity summary information of *L. geumgangensis* and *L. mediadiposalis*.

Species	Population Code	Water System	*N*	*h*	H_d	Nucleotide Diversity (π)	*D*	*F*
L. geumgangensis	LgJC	Geumgang River	20	2	0.337	0.00066	0.45727	1.985
L. geumgangensis	LgGJ	Geumgang River	20	2	0.100	0.00010	−1.16439	−0.879
L. geumgangensis	LgMG	Mangyeongang River	20	1	0.000	0.00000	-	-
L. geumgangensis	LgJC, LgGJ	Geumgang River	40	4	0.619	0.00271	1.90551	4.441
L. geumgangensis	LgMG	Mangyeongang River	20	1	0.000	0.00000	-	-
L. mediadiposalis	LmMJ	Geumgang River	19	3	0.292	0.00231	−0.28784	3.737
L. geumgangensis	LgJC, LgGJ, LgMG	Geumgang and Mangyeongang River	60	5	0.725	0.00319	1.88005	4.910

N: number of samples, *h*: number of haplotypes, H_d: haplotype diversity, *D*: Tajima's D values, *F*: Fu's Fs values, *D* and *F* ($p > 0.05$).

The *h* for the water system in the *L. geumgangensis* population was 1–4, the H_d was 0.000–0.619, and the π was 0.000–0.00271. *D* and *F* were negative and non-significant in the LgGJ population.

L. mediadiposalis showed haplotype diversity similar to that of LgJC, with an H_d of 0.292. For *L. mediadiposalis*, *D* was negative and *F* was positive.

At the species level (LgGJ, LgJC, LgMG) of *L. geumgangensis*, the total *h* was 5, H_d was 0.725, and π was high at 0.00319. *F* and *D* were both positive.

3.2. Population Genetic Structure

The F_{ST} values for *cytb* in mtDNA were 0.923–0.981, indicating a high degree of differentiation (Table 2). The Geumgang and Mangyeonggang River water system populations showed a considerably high genetic differentiation of 0.952 and 0.976, respectively. The populations (JC and GJ) of the Geumgang River showed a high genetic differentiation of 0.923.

Table 2. F_{ST} among four populations of *L. geumgangensis* and *L. mediadiposalis* according to the *cytb* gene datasets.

	LgJC	**LgGJ**	**LgMG**	**LmMJ**
LgJC	-	0.000	0.000	0.000
LgGJ	0.923	-	0.000	0.000
LgMG	0.952	0.976	-	0.000
LmMJ	0.976	0.980	0.981	-

Pairwise genetic differentiation significance level (upper diagonal); pairwise genetic differentiation of *cytb* in mitochondrial DNA (mtDNA) (below).

In the median-joining network, the haplotypes were divided into two groups centered on H1 and H5 (Figure 2). Haplotypes H1 and H2 vs. H3 and H4 of the two Geumgang River populations (LgGJ and LgJC) were not shared with each other. The H8 haplotype was unique to the LgMG population. Minor differences were observed between the LgMG (H8) and LgGJ populations (H1), with only two sequence differences. H1, H3, and H4 showed six sequence differences and displayed additional differences compared with the Mangyeonggang River water system.

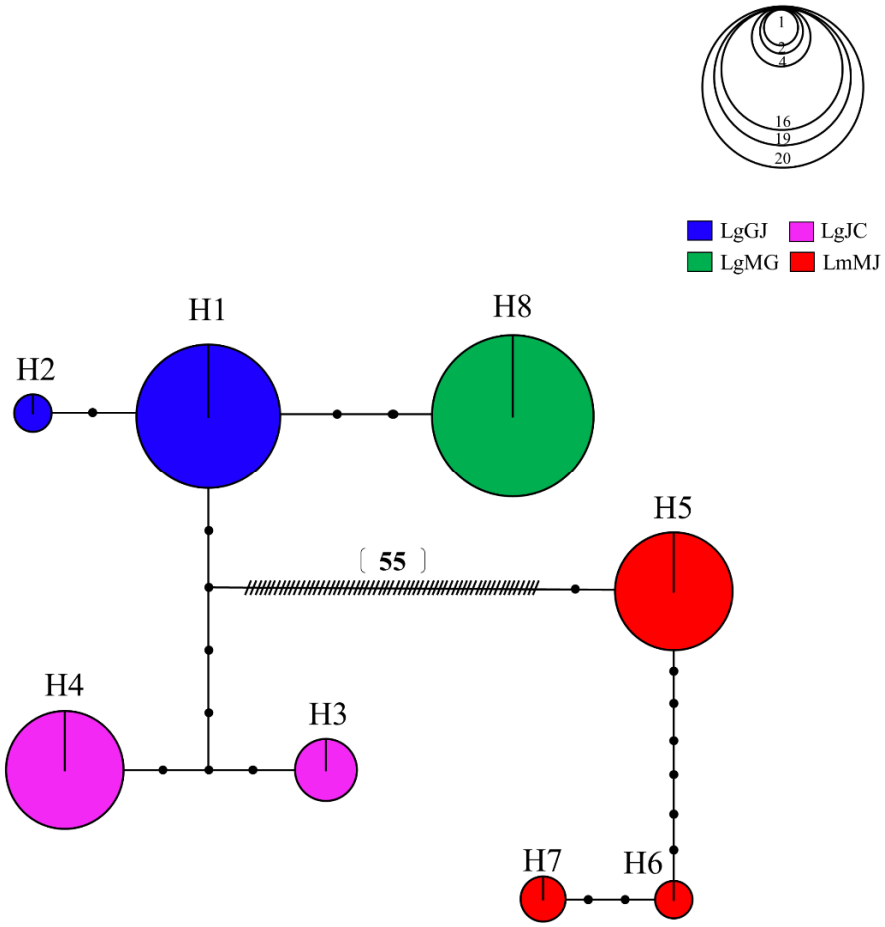

Figure 2. A haplotype network of four populations using mitochondrial *cytb* in *L. geumgangensis* (LgGJ, LgJC, and LgMG) and *L. mediadiposalis* (LmMJ). A single slash represents the mutant sequence, equivalent to a dot.

STRUCTURE results were analyzed in two groups (first group: *L. geumgangensis* and *L. mediadiposalis*, second group: *L. geumgangensis*), and the *K* constant of the first group was found to be 2 (Figure 3). The *K* constant of the second group was found to be 2, but it also showed high delta *K* values at *K* = 3. The first group was *L. geumgangensis* vs. *L. mediadiposalis* (LgGJ, LgMG, LgJC vs. LmMJ), whose genetic structure was divided into two. The second group was LgGJ, LgMG vs. LgJC, and the genetic structure was divided into two.

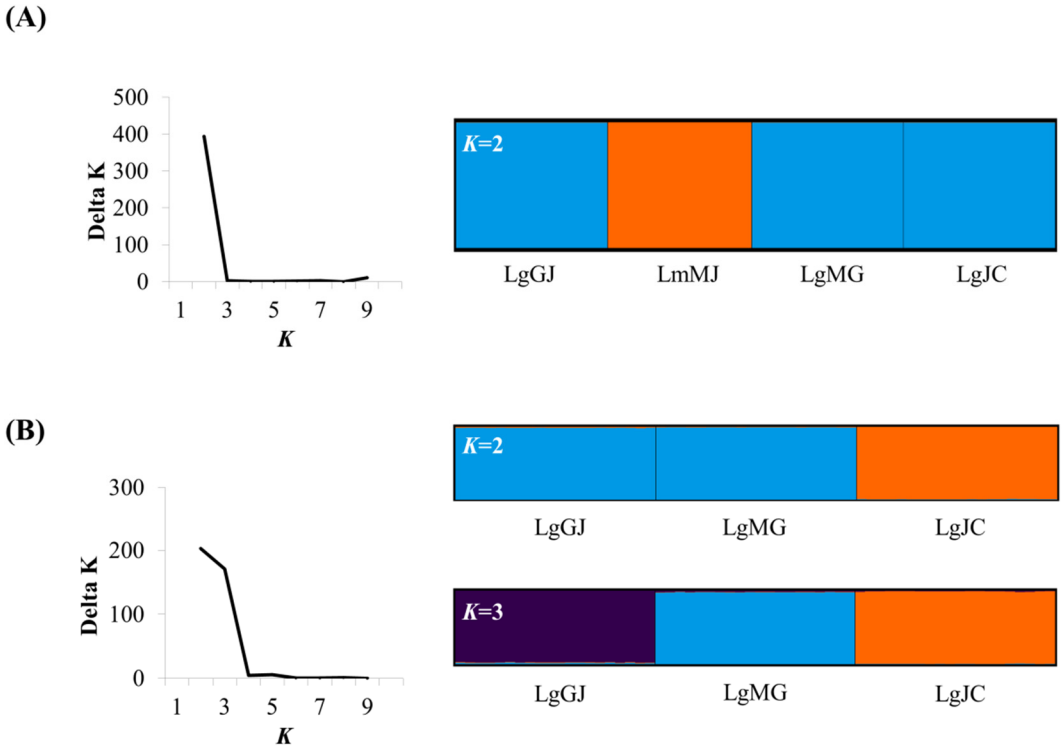

Figure 3. The figure shows the genetic structure graph corresponding to the Delta *K* value and the appropriate *K* value. (**A**): The first group is a genetic structure graph including *L. geumgangensis* and *L. mediadiposalis*. (**B**): The second group contains genetic structure graphs containing only *L. geumgangensis*.

4. Discussion

Our goal was to reveal the genetic structure of *L. geumgangensis* inhabiting the Geumgang and Mangyeonggang Rivers and to recommend conservation strategies through a comparison of the genetic diversity and structure with *L. mediadiposalis* newly discovered in the Geumgang River. We initially used the mtDNA *cytb* region to analyze the genetic diversity of three populations (LgJC, LgGJ, and LgMG) living in the Geumgang and Mangyeonggang Rivers and a population of *L. mediadiposalis* found in the upper Geumgang River (LmMJ). High mutation rates in the *cytb* region demonstrate the presence of polymorphic sites and haplotypes in the LgGJ and LgJC populations [27,28]. No shared haplotypes existed among the three populations (LgJC, LgGJ, and LgMG).

There are no reports of species-level genetic diversity within Amblycipitidae. Therefore, the genetic diversity within Siluridae was investigated, revealing that *L. geumgangensis* (H_d = 0.725) showed lower genetic diversity than *Silurus asotus* (H_d = 0.948) [29]. Genetic diversity is an important factor in determining the evolutionary potential of a species and its ability to respond to change [30]. Low genetic diversity can lead to the loss of evolutionary potential owing to genetic drift and the accumulation of deleterious alleles [8]. Low genetic diversity can make a species vulnerable to extinction, as in the case of *Anaecypris hispanica* and *Ladigesocypris ghigii* [31,32]. Habitat destruction and overfishing were not always observed to reduce genetic diversity in the short term [33]. This is probably

because short-term habitat destruction does not occur rapidly enough to induce biases in genetic variation among descendant generations. Therefore, the considerably low genetic diversity is assumed to be a long-term phenomenon. In particular, the Mangyeonggang River population may be more vulnerable to extinction owing to its low haplotype diversity. As they evolved recently and have a short evolutionary history, they may show low genetic diversity. Therefore, further research is needed to determine whether genetic diversity is low using nuclear DNA markers.

L. geumgangensis is an endemic species of the Geumgang and Mangyeong Rivers [1]. Currently, these rivers face a threat as shoals and gravel disappear because of habitat destruction caused by weirs and dams [1]. We performed neutrality tests on *D* and *F* statistics to investigate the history of each population. The values of *D* and *F* were negative in LgGJ; however, they were not statistically significant. Therefore, LgGJ did not experience population expansion or bottlenecks (LgGJ: $D = -1.16439$, $F = -0.879$). The reduction in genetic variation in wild populations owing to demographic bottlenecks is an important factor in conservation [34]. There has been no significant expansion in the Geumgang population; despite this, low genetic diversity persists, possibly contributing to its historically low genetic diversity. The lack of genetic diversity in this species may be a historical factor, not necessarily owing to recent demographic changes [35]. The *L. geumgangensis* Geumgang population did not appear to have undergone expansion, suggesting that its historical genetic diversity was low. However, this low historical genetic diversity does not imply genetic health, raising concerns about potential vulnerability to extinction.

According to the haplotype network and STRUCTURE results ($K = 2$), *L. geumgangensis* and *L. mediadiposalis* showed distinct genetic differences. This is believed to be a clear distinction owing to the large genetic differences between the species [1]. As a new *L. mediadiposalis* population was discovered in the Geumgang River, this study required information on the genetic diversity and structure of *L. mediadiposalis*. This was necessary because measurements of the genetic diversity and structure of *L. mediadiposalis* may pose ecological threats to *L. geumgangensis* by processes such as hybridization and competition.

Haplotype network analysis for *L. geumgangensis* showed a clear distinction between the Geumgang and Mangyeonggang River populations (LgJC vs. LgGJ vs. LgMG). The objective results of population clustering and segmentation showed high delta *K* values at $K = 2$ and $K = 3$ in STRUCTURE [23]. Therefore, the overall results are divided into three populations. The LgJC (Jicheon Stream) and LgGJ (Yongsucheon Stream) populations are tributaries flowing into the Geumgang River. LgJC and LgGJ are in the lower reaches and are located downstream of the Daecheong Dam, where the Geumgang River is divided into the upper, middle, and lower parts. The two Geumgang populations exhibited no shared haplotypes according to F_{ST} and haplotype network analyses. Because populations are unlikely to move long distances, it is assumed that there is no genetic exchange between populations. *Liobagrus* protects its spawning grounds through mating behaviors between males and females [35]. Therefore, 1:1 mating is preferred over 1:many mating [36]. *L. geumgangensis* is presumed to exhibit ecological behavior similar to *Liobagrus*, including protecting spawning grounds. However, the haplotype network results suggested limited genetic exchange and low haplotype diversity, concluding that the gene pool was small. However, the small sample size may have led to an interpretation bias. A possible reason for this could be that the gene pool may be biased because of the simultaneous sampling of fry from the same year. However, this possibility is low because the focus of the study was on adult fish, and multiple samplings occurred within a single location. Another possibility is that the limited mobility of *L. geumgangensis* results in individuals born in one location being sampled through dispersal. Because of their low mobility, they tend to remain in proximity until adulthood. If their habitat, characterized by gravel and shoals, is disrupted, it poses a risk to unique genotypes within populations, presenting challenges from a conservation genetics perspective. Future research is required to develop microsatellite markers and identify their genetic structure. However, the current study has already indicated a limited gene pool because of few haplotypes.

L. geumgangensis is an endemic species of the Geumgang and Mangyeonggang Rivers [1]. An *L. mediadiposalis* population (LmMJ) was discovered in Muju-namdaecheon in the upper reaches of the Geumgang River. The *cytb* sequence of this discovery was highly consistent (99.71% BLAST results) with *L. mediadiposalis* (OP980987) residing in the Nakdonggang River. It is possible that *L. mediadiposalis* from the Nakdonggang River was introduced into the Geumgang River or was originally native to the Geumgang River. If this is so, determining the haplotype genotype of the population becomes crucial because they will possess identical mtDNA. The BLAST results were not 100% consistent, thereby lacking conclusive evidence of introgression. However, two genetic mutations suggest a potential origin from similar populations. If translocation occurs, upstream *L. mediadiposalis* will be pushed out through habitat competition with *L. geumgangensis* located downstream owing to flooding, which may also cause hybridization.

The introduction of *L. mediadiposalis* poses a potential threat to its persistence because introgression with weak reproductive barriers could occur. Similar threats owing to hybridization following introductions are documented in other taxa [33]. Both hypotheses may be unfavorable for *L. geumgangensis* from a conservation genetics perspective, suggesting the presence of biological and physical threats. These threats should be further explored using distributional and ecological surveys of *L. geumgangensis*. Therefore, we believe that this study will contribute to the conservation of *L. geumgangensis* by investigating its genetic structure using mitochondrial *cytb*, thereby generating ecological and genetic interest for the conservation of *L. geumgangensis*.

5. Conservation Implications

L. geumgangensis is an endemic fish species inhabiting clear waters with shoals and pebbles [1]. However, the genetic diversity and structure of *L. geumgangensis* populations have not been elucidated. Therefore, investigating genetic diversity and structure is crucial as basic data for the conservation and preservation of the *L. geumgangensis* species [35,36]. In this study, the genetic diversity of *L. geumgangensis* was found to be lower than that of other freshwater fish populations. The low genetic diversity of the population makes it susceptible to gradual decline, especially in the face of escalating threats like river dam construction and habitat destruction. These species may face extinction if active conservation efforts are not undertaken. Specifically, *L. mediadiposalis* populations were identified in the upper reaches of the Geumgang River population. Therefore, the populations of tributaries (LgJC, LgGJ) flowing into the river should be designated as conservation areas to prevent the possibility of the interbreeding of *L. geumgangensis* and prevent them from being pushed out ecologically. Considering the low mobility of the species, each microhabitat requires designation and management as an individual conservation area. Additionally, the genetic diversity of the current downstream Geumgang River population must be maintained. However, since the downstream dispersal of *L. mediadiposalis* due to flooding cannot be prevented, an ecological distribution survey is needed to identify the exact habitat. Furthermore, conservation efforts should include genetic monitoring of the identified habitat to ensure the preservation of the species. Conservation efforts may need to focus on maintaining the species within aquaculture in a dedicated out-of-habitat conservation institution if preventing the spread of *L. mediadiposalis* proves challenging.

Conservation strategies should be planned according to genetic structure [8]. In the *cytb* region of mtDNA, the Geumgang (LgGJ and LgJC) and the Mangyeonggang River populations (LgMG) each had unique haplotypes and genotypes that were genetically distinct. Therefore, each population must be treated as a distinct conservation area. The importance of separately managing them arises from the uncertainty regarding the potential effect of outbreeding on species persistence. As outbreeding can lead to outbreeding depression [29], additional research is necessary to develop independent microsatellite markers in the future. Our results provided valuable information on the genetic basis for the conservation of *L. geumgangensis*.

6. Conclusions

With respect to the conservation of *L. geumgangensis*, it has been noted that *L. mediadisposalis* was found in the upper reaches of the Geumgang River. Therefore, it is recommended to designate the populations of tributaries (LgJC, LgGJ) flowing into the river as protected conservation areas to prevent the hybridization of *L. geumgangensis* and its ecological displacement. If controlling the spread of introduced *L. mediadiposalis* proves challenging, it becomes imperative to implement conservation measures within fish farms. This may involve collaboration with non-habitat conservation agencies to ensure the preservation of species. In the *cytb* region of mtDNA, the two Geumgang populations (LgGJ vs. LgJC) and the Mangyeonggang population (LgMG) had unique haplotypes and genotypes that were genetically different. Therefore, each population was treated as a distinct conservation area. Additional research on the development of independent microsatellite markers is necessary in the future. Our results provide information on the genetic basis for the conservation of *L. geumgangensis*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9050153/s1, Table S1: Sampling sites and number of individuals in the study.

Author Contributions: Conceptualization, K.-R.K. and K.-S.K.; methodology, K.-R.K. and M.-S.S.; software, K.-R.K.; validation, K.-R.K.; data curation, K.-R.K.; writing—original draft preparation, K.-R.K.; writing—review and editing, K.-R.K.; supervision, K.-R.K. and K.-S.K.; project administration, K.-S.K.; funding acquisition, K.-S.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the guidelines and regulations of the National Institute of Ecology. All experiments were performed in accordance with ARRIVE-related guidelines and regulations of the National Institute of Ecology. Population samples were collected according to the guidelines set out by the National Institute of Ecology. No permission from the government was required to collect the fish. The authors comply with the relevant institutional, national, and international guidelines and legislation for fish studies. Ethics Committee Name: National Institute of Ecology, Approval Code: NIE-01, Approval Date: 1 January 2023.

Data Availability Statement: The *cytb* sequence was deposited in GenBank (PP061219–PP061226).

Conflicts of Interest: The authors declare no conflicts of interest.

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