ASSESSMENT OF GENETIC VARIATION IN THE POND LOACH *MISGURNUS ANGUILLICAUDATUS* (CANTOR, 1842), BASED ON *CO*I GENE FRAGMENTS

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Abstract. The pond loach *Misgurnus anguillicaudatus* is a freshwater fish of the loach family Cobitidae, which belongs to the order Cyrpiniformes. The fish contains high nutritional and medicinal values. In Vietnam, they are mainly distributed in the Northern to Central and Tay Nguyen areas. In order to study the genetic diversity and population structure of *M. anguillicaudatus* populations in Central Vietnam, partial sequences of cytochrome c oxidase subunit I gene from 48 individuals were used to analyze. We detected high levels of genetic diversity in four *M. anguillicaudatus* populations (Hd = 0.889 ± 0.032, π = 0.010 ± 0.003). Analysis of molecular variance identified 13.64% variance among and 86.36% variance within populations. The fixation index value was 0.13635 with the probability value < 0.000001, showing the presence of moderate-to-significant genetic differentiation. The current study provides valuable genetic diversity data and the population structure of *M. anguillicaudatus* are meaningful to scientist and industrial communities in the field of fish conversation and genetic resource utilization.
Keywords: *genetic diversity, population structure, molecular variance, fixation index, Central Vietnam*

Introduction

The pond loach *Misgurnus anguillicaudatus* (Cantor, 1842) (Cobitidae: Cyrpiniformes) is a native freshwater fish found from East to Southeast Asia, including Myanmar, Cambodia, Laos, Vietnam, China, India, Japan, Thailand, Korea, and Taiwan, (Simon et al., 2006). Due to high economic values, *M. anguillicaudatus* has been cultivated in various countries such as Belgium, Austria, America, Australia, South America, Italy, Netherlands, Spain (Wanzenböck et al., 2021); Germany (Freyhof and Korte, 2005); Brazil (Gomes et al., 2011); Palau, Mexico, Northern Africa (Van Kessel et al., 2013); the Iberian Peninsula (Franch et al., 2008). The main economic contribution of the pond loach is varied, including use as a food source (Park et al., 2006), an ornamental fish for the aquarium trade (Strecker et al., 2011), an object for aquaculture and fisheries, and a baitfish (Franch et al., 2008). In addition, *M. anguillicaudatus* has also been cultivated in several other localities, probably due to escape from fish farms and ponds or release by aquarists (Freyhof et al., 2005).

This small benthic fish species lives in rivers, streams, ditches, ponds, lakes, swamps, and rice paddy fields (Hao, 2005). Shallow and calm water with a sandy or muddy bottom is a habitat that they prefer (Tran et al., 2017). In Vietnam, the pond loach fish (in their native habitats) can be found in the Northern, Central, and Tay Nguyen regions (Hao, 2005).

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The meat of the pond loach is delicious, tender, flavorful, rich in nutrients, and also has a high medical value (Dong et al., 2002). Numerous Vietnamese dishes can be processed from the pond loach such as crispy fried loach, grilled loach, caramelized loach, steamed loach with lemongrass, loach hotpot, loach soup, loach braised with noni leaves or pepper, and loach cooked with fermented rice or water lilies. In traditional Chinese medicine, the pond loach has been used for the treatment of some diseases including inflammations, hepatitis, carbuncle, and cancers (Wang et al., 2009; Dou et al., 2023), particularly diabetes (Dong et al., 2002).

Various healthful, active substances obtained from *M. anguillicaudatus* have been demonstrated to have high pharmacological values, such as antioxidant peptides (You et al., 2009), antimicrobial polypeptides including misgurin (You et al., 2010), polypeptides from the whole body homogenates of loach (You et al., 2011a); antioxidative peptides (Wang et al., 2009) and crude peptides with antifatigue, anticancer cell proliferative effects (You et al., 2011) from the loach meat. *M. anguillicaudatus* polysaccharide (MAP) exhibited antioxidant bioactivity, antiproliferative and apoptotic effects on tumor cells (Zhang and Huang, 2006). On the other hand, lectin (Sun et al., 2019) and glycoprotein (Nakagawa et al., 2001) have been extracted from the loach skin mucus for food and medicinal applications. Thus, the pond loach is one of the species with a high market value. Because of overexploiting, the number of natural wild *M. anguillicaudatus* has sharply decreased. Therefore, information on genetic diversity and population structure of wild loach stocks is particularly important for developing appropriate management and conservation policies.

In recent years, many different genetic markers have been used for the genetic investigation of *M. anguillicaudatus* such as cytochrome b gene (*cyt b*) (Ke et al., 2002; Yang et al., 2009), control region (CR) (Kano et al., 2011), microsatellite (Abbas et al., 2017), RAG1 gene (Zangl et al., 2020), single nucleotide polymorphisms (SNPs) (Yi et al., 2019), restriction fragment length polymorphism (RFLP) (Morishima et al., 2008), randomly amplified polymorphic DNA (RAPD) (Morishima et al., 2008), and cytochrome oxidase gene subunit I (*CO*I) (Belle et al., 2017). The *CO*I gene, a region that encodes proteins in a large number of copies in cells, is commonly used in genetic species identification, phylogenetic tree analysis, genetic diversity, history evolution, and population genetics in animals (Hebert et al., 2003), especially for fish (Imtiaz et al., 2017). The *CO*I barcoding exhibited high-efficiency and perfect barcode for fish species identification, and it has been used to identify 194 freshwater fish species in Canada with a success rate of 98% (Hubert et al., 2008); 64 fish species in the Itapecuru Basin in Maranhão, Brazil with a success rate of 92.19% (Nascimento et al., 2016); 100 specimens of fish larvae with a success rate of > 65% at the species level (Ko et al., 2013); yellowfin tuna (*Thunnus albacares*) (Higashi et al., 2016), and Atlantic goliath groupers (*Epinephelus itajara*) (Damasceno et al., 2016); *Anguilla* eels (Huyen et al., 2020). Imtiaz et al. (2017) showed that *CO*I barcode data could provide partial information about the phylogeny of species and can draw an outline for phylogeny. Recently, the *CO*I gene has been used to identify 115 Indian marine fish species that were grouped into 79 genera when the NJ phylogenetic tree was constructed to study the phylogenetic relationship between collected specimens (Lakra et al., 2011); 21 Amazonian commercial fish species were obtained at a specific level in the reconstructed NJ phylogenetic tree with 100% bootstrap support (Ardura et al.,, 2010); to analyze phylogeny of *Anguilla marmorata* population (Huyen and Linh, 2020); to examine genetic diversity within and among 85 fish species in the Taiwan Strait

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(Bingpeng et al., 2018). In Vietnam, the study of the genetic diversity of *M. anguillicaudatus* by RAPD was reported (Tran et al., 2017). Until now, there has been no analysis of the genetic diversity of *M. anguillicaudatus* natural populations by *CO*I sequence.

The objectives of this study are to provide the first data on genetic diversity and population structure of the pond loach in Vietnam for application in bio-conservation and management of aquaculture resources as well as contribute to the *CO*I nucleotide sequences database of loaches. By analyzing the sequences of *CO*I genes for 48 individuals, the genetic diversity and the population structure of *M. anguillicaudatus* in the areas were determined.

Materials and methods

Sample collection

Live specimens of pond loaches were collected from some provinces of Central Vietnam in January 2023 (*Fig. 1*): Hue, Quang Tri (QT), Quang Binh (QB), and Ha Tinh (HT) (*Table 1; Fig. 2*). All the specimens were confirmed morphologically based on the taxonomic key (Yen, 1978). After cutting a very small caudal fin sample (~2 g) off, the fish still lived normally, and they were released into their natural habitat. A total of 48 caudal fin samples of pond loaches *M. anguillicaudatus* were collected and preserved in 70% ethanol at 4°C for DNA analysis.

Table 1. *Specimens M. anguillicaudatus with locality and voucher code*

Locality	Number of specimens	Voucher code
Hue	11	From Hue01 to Hue11
QT	9	From QT01 to QT10
QB	15	From QB01 to QB15
HT	13	From HT01 to HT13
Total	**48**	

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from each stored caudal fin sample using TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam) following the manufacturer's recommendations. The quality of extracted DNA was estimated by electrophoresis on 0.8% agarose gel and measuring its absorbance value by photo-spectrometer (optical density at 260 nm/280 nm ratio). The extracted DNA solutions were stored at -20°C until analysis. The polymerase chain reaction (PCR) was performed using primer pair: FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR2: 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3' (Ward et al., 2005) for amplification of the 629 bp partial *CO*I genes. The amplification reaction was done in a final volume of 50 µL, containing 25 µL 2× Go Taq® Green Master Mix (M7502, Promega, USA), 20 pg each primer, 100 ng DNA template, and ultrapure water to the final reaction volume. The amplification reaction was set as below: pre-denatured for 10 min at 95°C; denatured for 1 min at 95°C, annealed for 1 min at 57°C, extended 10 min at 72°C for

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30 cycles; again extended for 10 min at 72°C. The PCR products were visualized on 0.8% agarose gels. Sequencing reactions were performed by First BASE Laboratories Sdn Bhd (Selangor, Malaysia). Newly obtained sequences were deposited in the NCBI GenBank database with the registered accession numbers OQ553874-OQ553921.

Figure 1. *Pond loach M. anguillicaudatus (Cantor, 1842)*

Figure 2. *Sampling localities of pond loach M. anguillicaudatus*

Data analysis

The nucleotide sequences were manually edited and aligned using BioEdit software. Basic Local Alignment Search Tool (BLAST) was used for similarity searching of the *CO*I sequences in GenBank (http://blast.ncbi.nlm.nih.gov/). The average base

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composition was calculated using GC Content Calculator (https://www.biologicscorp.com/tools/GCContent/).

The number of haplotypes, haplotype diversity (Hd), number of mutations (η), nucleotide diversity (π), number of polymorphic sites (S), the average number of nucleotides differences (k), Tajima's D and Fu's Fs values were calculated using DnaSP v6.12 software (Rozas et al., 2017). Population expansion patterns of pond loach *M. anguillicaudatus* populations were evaluated by estimating Tajima's D and Fu's Fs tests. Fu's Fs test is based on the distribution of haplotypes, while Tajima's D test is based on the allele frequency when comparing pairwise differences between the sequences (Ramírez-Soriano et al., 2008). MEGA11 software was used to calculate genetic distances and reconstruct the phylogenetic tree using the Maximum Likelihood model based on the obtained distance matrix. The confidence level of the phylogenetic tree was tested by the bootstrap method with 1000 replicates (Tamura et al., 2021). For phylogenetic analysis, 21 additional *CO*I sequences of *M. anguillicaudatus*, *Paramisgurnus dabryanus*, *Misgurnus bipartit*e, and *Misgurnus fossils* were obtained from GenBank using as outgroups (*Table 2*). Analysis of molecular variance (AMOVA) was performed to obtain the genetic differentiation indices (F_{ST}) and genetic variation partitioning within and among populations using the population genetics package Arlequin 3.5.2 (Excoffier and Lischer, 2010). Gene flow (Nm) (Hudson et al., 1992) was analyzed by DnaSP v6.12 software (Rozas et al., 2017).

Table 2. *Species, sampling location, GenBank accession numbers of individual COI sequences used in this study*

Species	Sampling location	GenBank accession numbers
Misgurnus anguillicaudatus	USA, New York	MT667249
Misgurnus anguillicaudatus	Central China Mountains	MN913480
Misgurnus anguillicaudatus	Korea	EU670785
Misgurnus anguillicaudatus	China, Hubei, Wuhan	KP112321.1
Misgurnus anguillicaudatus	Korea	MN709580
Misgurnus anguillicaudatus	Italy	KJ553886.1
Misgurnus anguillicaudatus	China	KM610758.1
Misgurnus anguillicaudatus	China, Yangtze River	MF122497
Misgurnus anguillicaudatus	China, Wujiang River	MZ870966.1
Misgurnus anguillicaudatus	Central China	KP112320
Misgurnus anguillicaudatus	Canada	KX224170.1
Misgurnus anguillicaudatus	Canada	KX224173.1
Paramisgurnus dabryanus	China	KM610790.1
Paramisgurnus dabryanus	China	KM610791.1
Paramisgurnus dabryanus	China	KM610792.1
Misgurnus bipartitus	Korea	MN709587
Misgurnus bipartitus	Northeast China	KX505273
Misgurnus bipartitus	Northeast China	KX505265
Misgurnus fossilis	Germany	KM286763.1
Misgurnus fossilis	Germany	KM286764.1
Misgurnus fossilis	Germany	KM286765.1

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Results

Genetic diversity of M. anguillicaudatus populations

A fragment of 629 bp of the *M. anguillicaudatus* mtDNA *CO*I gene was obtained from all 48 samples collected from the Central provinces of Vietnam. Among 48 sequences, 84 variable sites were observed, of which 53 were single variable sites, and 31 were parsimony-informative sites (*Table 3*). The average base composition was T = 33.37%, C = 25.50%, A = 23.86%, and G = 17.27%. The average T composition was the highest (33.37%), and the average G composition was the lowest (17.27%). All three codon positions experienced base-compositional biases towards A + T (57.23%) as compared with G + C (42.77%).

Twenty-four distinct haplotypes were defined, among which nine were obtained from 11 individuals of the Hue population, the QT population detected 7 haplotypes from 9 individuals, the QB population detected 7 haplotypes from 15 individuals, and 6 from 13 individuals of the HT population. All four populations had unique haplotypes, while one haplotype was shared by Hue, QT, and QB populations, and one haplotype was shared by Hue, QT, and HT populations. The Hd value ranges from 0.641 ± 0.150 to 0.945 ± 0.066 (mean = 0.889 ± 0.032) and the π value ranges from 0.002 ± 0.001 to 0.021 ± 0.004 (mean = 0.010 ± 0.003). The highest Hd and π values were found in samples from Hue (Hd = 0.945 ± 0.066 and $\pi = 0.021 \pm 0.004$, respectively), and the lowest Hd (0.641 ± 0.150) and π values (0.002 ± 0.001) found in samples from Ha Tinh (*Table 3*). The results indicated that the pond loaches *M. anguillicaudatus* in Central Vietnam exhibited high haplotype diversity and nucleotide diversity.

Table 3. *Genetic diversity of M. anguillicaudatus populations in Central Vietnam based on COI sequence*

Population	Number of samples	Number of haplotypes	Haplotype diversity (Hd ± SD)	Nucleotide diversity (π ± SD)	Number of variable Sites (S)	Number of mutations (η)	Average nucleotide differences (k)
Hue	11	9	0.945 ± 0.066	0.021 ± 0.004	50	51	13.036
QT	9	7	0.944 ± 0.070	0.016 ± 0.004	42	43	10.028
QB	15	7	0.838 ± 0.085	0.003 ± 0.002	12	12	2.057
HT	13	6	0.641 ± 0.150	0.002 ± 0.001	7	7	1.308
Total	48	24	0.889 ± 0.032	0.010 ± 0.003	84	91	6.392

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh

Population genetic structure of M. anguillicaudatus populations

AMOVA showed the percentages of variation among and within populations of *M. anguillicaudatus* populations in Central Vietnam were 13.64% and 86.36%, respectively. The F_{ST} value was 0.13635, with the p-value being < 0.000001 (*Table 4*). Similar results were obtained from F_{ST} analyses with F_{ST} values detected between populations, ranging from 0.01485 to 0.39335 (*Table 5*). Significant pairwise F_{ST} values were detected between Hue and QB ($F_{ST} = 0.14041$, $p < 0.05$), Hue and HT (0.18540, $p < 0.05$), HT and QT (0.13770, $p < 0.05$), and HT and QB (0.39335, $p < 0.05$) (*Table 5*). The values of Nm between the four populations varied from 0.39 (between HT and QB) to 18.10 (between QT and QB), with a mean value of 2.19 (*Table 6*).

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Table 4. *Analysis of molecular variance (AMOVA) results for M. anguillicaudatus populations collected in Central Vietnam*

Source	Degree of freedom	Sum of squares	Variance components	Percentage of total variance (%)
Among populations	3	23.639	0.46971 Va	13.64
Within populations	44	130.903	2.97506 Vb	86.36
Total	47	156.542	3.44477	100
Fixation index (F_{ST})	0.13635 Remark: Va and F_{ST} p-value < 0.000001 ± 0.000000			

Table 5. *F_{ST} values (above diagonal) and probability values (below diagonal) among M. anguillicaudatus populations collected in Central Vietnam*

Population	Hue	QT	QB	HT
Hue	---	0.01485	0.14041*	0.18540*
QT	0.22523	---	0.05319	0.13770*
QB	0.00901*	0.06306	---	0.39335*
HT	0.00000*	0.00000*	0.00000*	---

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh
*Significant at 5% level

Table 6. *Gene flow between M. anguillicaudatus populations collected in Central Vietnam*

Population	Hue	QT	QB	HT
Hue	---	-	-	-
QT	13.26	---	-	-
QB	1.97	18.10	---	-
HT	1.18	2.21	0.39	---

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh

Results of Tajima's D test and Fu's Fs test were presented in *Table 7*, including associated simulated p-values. The Tajima's D and Fu's Fs values were negative and not significant in almost all populations (except for QT).

Table 7. *Tajima's D and Fu's Fs with corresponding probability values in parentheses*

Population	Tajima's D	Fu's Fs
Hue	-1.186 (p > 0.10)	-0.263 (p > 0.01)
QT	-1.859* (p < 0.05)	0.288* (p < 0.05)
QB	-1.724 (0.10 > p > 0.05)	-2.976 (p > 0.01)
HT	-1.594 (0.10 > p > 0.05)	-2.317 (0.10 > p > 0.05)
Total	-2.452* (p < 0.01)	-7.410* (p < 0.02)

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh
*Significant at 5% level

The values of genetic identity among *M. anguillicaudatus* populations collected in Central Vietnam based on *CO*I gene fragments were calculated and given in *Table 8*. The results indicated that the values of genetic identity between populations were high,

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ranging from 99.52% to 99.81%. The populations originating from QB and HT had the highest genetic identity (99.81%), and the population pair Hue-QT had the lowest genetic identity (99.52%). These high values of genetic identity might reflect the low levels of genetic variability by different pond loach populations.

Table 8. *Genetic identity (%) (below diagonal) and genetic distance (%) (above diagonal) among M. anguillicaudatus populations collected in Central Vietnam*

Population	Hue	QT	QB	HT
Hue	---	0.48	0.36	0.39
QT	99.52	---	0.24	0.28
QB	99.64	99.76	---	0.19
HT	99.61	99.72	99.81	---

QT: Quang Tri; QB: Quang Binh; HT: Ha Tinh

Based on the sequence of *CO*I gene fragments of *M. anguillicaudatus* individuals in Central Vietnam and the reference sequences taken from GenBank (*Table 2*), a phylogenetic tree was built using Maximum Likelihood analyses (*Fig. 3*). The phylogenetic tree showed that *M. anguillicaudatus* from Central Vietnam were grouped in one cluster, which reflected the prior taxonomic assignment based on morphology. Meanwhile, *M. anguillicaudatus* from Korea, Italy, China, Canada, and the USA from GenBank were grouped in the other cluster.

Discussion

The *CO*I gene is the genetic marker most utilized in fish DNA barcoding studies, and it has been reported with high efficiency for species identification and genetic diversity of pond loach *M. anguillicaudatus* in Austria (Zangl et al., 2020); Southern Germany (Belle et al., 2017); Yangtze River Basin, China (Yi et al., 2019). The present study examined the genetic diversity and population genetic structure of pond loach *M. anguillicaudatus* in Central Vietnam based on mitochondrion *CO*I sequences.

In eukaryotes, co-amplifying nuclear mitochondrial pseudo-genes (NUMTs) and mitochondrial genes frequently occur which generates errors during the mtDNA nucleotide sequencing. Typically, the errors are presented if the amplicon size is below 600 bp (Imtiaz et al., 2017; Zhang and Hewitt, 1996; Ward et al., 2005). In our study, the size of all the amplified *CO*I sequences was 629 bp which is larger than 600 bp, indicating co-amplifying of NUMTs was eliminated. Moreover, there was no evidence of NUMTs co-amplifying in the fish (Bingpeng et al., 2018). Thus, the *CO*I gene sequences of the pond loach were free of NUMTs.

Genetic diversity

Genetic diversity is the variation in genes that occur within a species. Genetic diversity is important because it enables species to adapt to the varying changes in the environment. High levels of genetic diversity are indicative factors of the strong viability and adaptability of species (Barrett and Schluter, 2008). Analysis of the partial *CO*I sequences of *M. anguillicaudatus* populations in Central Vietnam found 24 distinct haplotypes and 84 polymorphic sites detected from 48 samples (*Table 3*).

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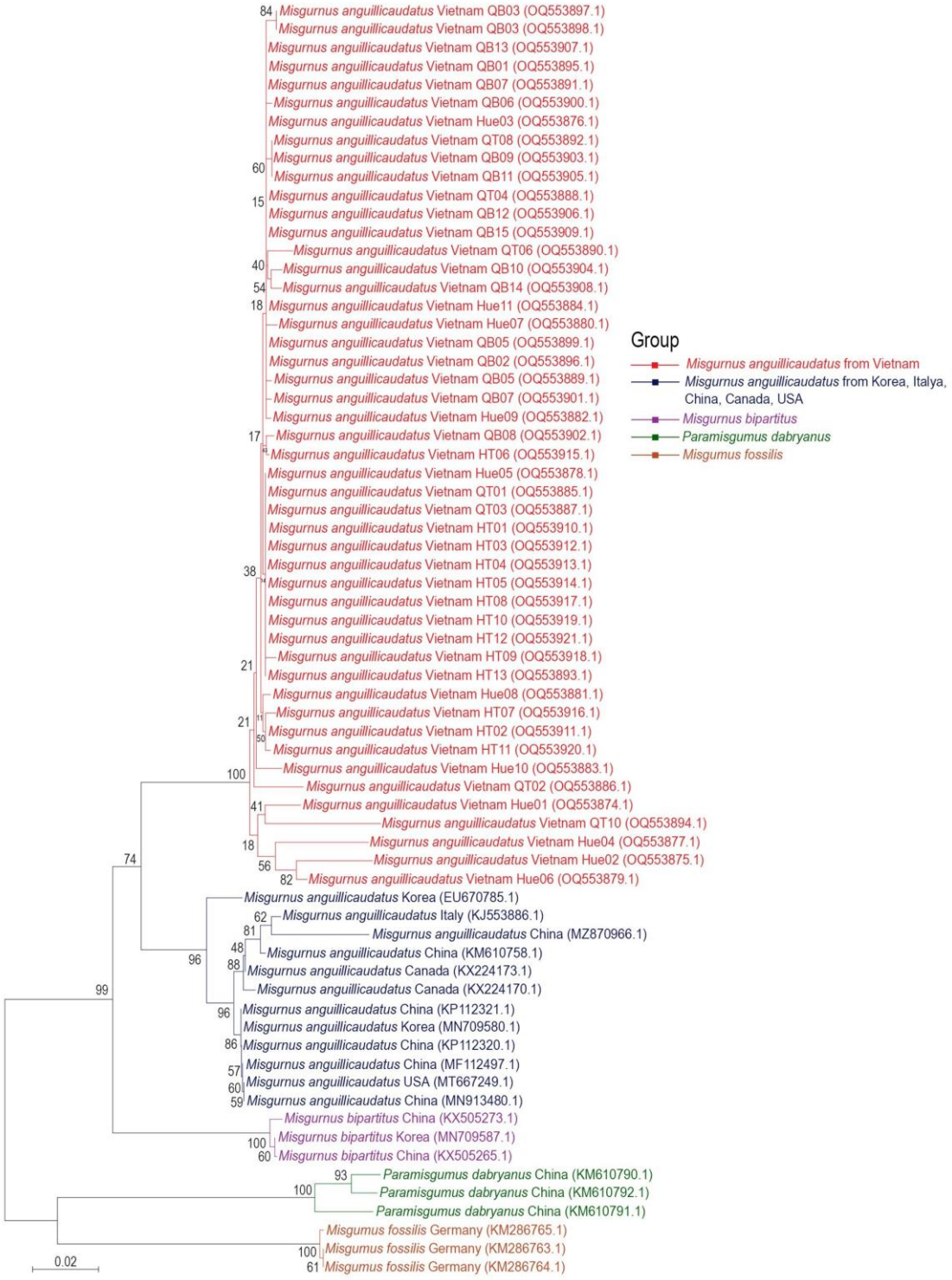

Figure 3. *The Maximum Likelihood phylogenic tree of pond loach M. anguillicaudatus in Central Vietnam based on COI sequences. The numbers on the branches are bootstrap values in percentage of 1000 replications. Numbers in parentheses represent the current GenBank accession numbers*

The base composition analysis of the *CO*I sequence revealed A + T content (57.23%) was higher than the G + C content (42.77%), showing a strong A/T bias. This result is

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consistent with the results found in *Gymnocypris przewalskii* (A + T = 57.33%), *Schizothorax biddulph* (A + T = 53%, 53.1%), and *Schizothorax irregularis* (A + T = 53.2%) (Fang et al., 2022). In addition, our result is similar to the results found in the sequence composition of the mitochondrial genome of *M. anguillicaudatus* in the Poyang Lake (A + T = 58.00%) (Zhang et al., 2020), Nansi Lake (A + T = 58.00%) (Zhang et al., 2019), Taihu Lake (A + T = 58.00%) (Zhang et al., 2018); the *cyt b* gene of *M. anguillicaudatus* in Central China (A + T = 58.90%) (Yang et al., 2009).

In the *CO*I sequences analysis, Hd and π are two crucial parameters used to assess genetic diversity. Hd > 0.5 and π > 0.005 indicate a high degree of genetic diversity (Grant and Bowen, 1998). Based on the *CO*I gene of four *M. anguillicaudatus* populations, the parameters Hd = 0.889 (>0.5) and π = 0.010 (>0.005) were consistent with the results found in *M. anguillicaudatus* in the Pearl River Basin based on *cyt b* gene (Hd = 0.9250 and π = 0.0539), and CR (Hd = 0.8790 and π = 0.0160) (Ke et al., 2022). These results suggested that *CO*I sequences revealed abundant genetic diversity of *M. anguillicaudatus* natural populations in Central Vietnam. Furthermore, similar results were detected for *M. anguillicaudatus* in Vietnam, showing high genetic diversity using RAPD markers (Tran et al., 2017).

Population genetic structure

Genetic differentiation among the populations can be inferred from Va (variance component) and average F_{ST} values on AMOVA results. The results show that the populations of *M. anguillicaudatus* in Central Vietnam have a Va value of 0.46971 and an F_{ST} value of 0.13635 with the probability value (p-value) < 0.000001. The observed p-value < 0.000001 ± 0.00000 for both Va and average F_{ST} values indicates significant genetic variation among *M. anguillicaudatus* populations in Central Vietnam.

F_{ST} values typically range from 0 to 1.0. $F_{ST} = 0$: no genetic differentiation; $0 < F_{ST} < 0.05$: little genetic differentiation; $0.05 < F_{ST} < 0.15$: moderate genetic differentiation; $0.15 < F_{ST} < 0.25$: significant genetic differentiation; $0.25 < F_{ST} < 1.0$: highly significant genetic differentiation; $F_{ST} = 1.0$: completely genetic differentiation (Khan et al., 2021). Herein, the F_{ST} value was 0.13635 ($0.05 < F_{ST} < 0.15$), and the pairwise estimates of F_{ST} indicated the presence of moderate-to-significant genetic differentiation between the loach populations. The highest level of differentiation was found as 0.39335 among HT and QB, while the lowest F_{ST} value of 0.01485 was observed for QT and Hue (*Table 5*). This result is in agreement with the results found in *M. anguillicaudatus* populations in middle-and-lower reaches of the Yangtze River basin, China, based on microsatellite ($F_{ST} = 0.090$, $p < 0.001$) (Abbas et al., 2017). Nevertheless, previous studies found high levels of genetic differentiation among *M. anguillicaudatus* populations based on microsatellite ($F_{ST} = 0.3395$) (Ke et al., 2022); *cyt b* gene ($F_{ST} = 0.2529$) (Yang et al., 2009). Additionally, most genetic variation resulted from the difference within populations (86.36%) (*Table 4*), indicating a high gene exchange between the four populations. This result is consistent with those detected for *M. anguillicaudatus* in China: 90.50% (Abbas et al., 2017).

Another important parameter used to evaluate the genetic structure of a population is Nm, which mentions the number of migrants per generation. The values of Nm were divided into three grades: $Nm \geq 1.0$: high; $0.25 < Nm < 0.99$: medium; and $0 < Nm < 0.249$: low (Jin et al., 2020). In this study, the mean Nm of the four pond loach populations was greater than 1.0 (2.19), indicating that the level of genetic communication among the four *M. anguillicaudatus* populations was high. This result is

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higher than that of the populations in the Pearl River Basin (Nm = 0.4863) (Ke et al., 2002), and in the Yangtze River Basin (Nm = 1.63) (Abbas et al., 2017) based on microsatellites. The results also showed high levels of gene flow among different populations (Nm varied from 1.18 to 18.10), except for the medium level of gene flow between QB and HT populations (N = 0.39). On the other hand, the level of genetic exchange between HT and QB, HT and QT, HT and Hue populations were lower than those of between QB, QT, and Hue populations each other (*Table 6*). This finding indicated the Ngang Pass (a mountain pass on the border of the QB and HT provinces) is a natural barrier to gene flow.

All populations except QT showed negative values of Tajima's D (*Table 7*) but were not statistically significant, indicating an excess of rare genetic variants, consistent with positive selection. Similarly, negative values of Fu's FS test in almost populations (except for QT) (*Table 7*) indicate an excess of rare haplotypes. Following Fu's Fs test, the hypothesis of neutral evolution was significantly rejected for QT. The negative values result in both neutrality tests indicating an excess of rare mutations in the populations of *M. anguillicaudatus*, but the excess is not statistically significant. All the *M. anguillicaudatus* species from Central Vietnam were clustered into monophyletic units in the phylogenetic tree, indicating that the *CO*I barcode has high efficiency in species identification (*Fig. 3*).

Conclusions

The present study evidenced that the genetic diversity level of pond loach *M. anguillicaudatus* in Central Vietnam was high. Moderate-to-significant genetic differentiation was recorded, and most of the genetic variation was found within populations. The population genetics of pond loach *M. anguillicaudatus* was important in revealing its situation in the natural environment. The present results also accumulate basic genetic data of pond loach *M. anguillicaudatus* for the conversation and its genetic resource utilization.

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