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Population structure and mitogenomic analyses reveal dispersal routes of *Macrobrachium nipponense* in China

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Abstract

Background The oriental river prawn *Macrobrachium nipponense* is widely distributed in China, but its origin and distribution routes remain largely unknown. We collected 126 oriental river prawn specimens from four lakes and one river across China, and sequenced their mitochondrial cytochrome C oxidase subunit I (*cox1*) genes. We performed whole-genome resequencing of 100 samples and assembled mitogenomes for population analysis, these two types of mitochondrial markers (*cox1* and all 13 protein-coding genes—13 PCGs), a nuclear marker (28S rRNA) and SNPs to infer the relationships between the five populations, the population structure, and migratory routes. We also assembled complete mitogenome per sampled population (5 in total) and used them to conduct comparative mitogenomic analyses.

Results The complete mitogenomes comprised 15,774—15,784 base pairs (bp). The average nucleotide diversity (π) of the populations, inferred using the *cox1* gene data, was 0.03013 ± 0.00618, ranging from 0.00500 ± 0.00110 (Fuxian Lake) to 0.03562 ± 0.02538 (Khanka Lake). The identified haplotypes (33 *cox1* and 101 13 PCGs) clustered into three main geographical lineages. Lineage A included Khanka Lake and one clade from the Haihe River. The specimens from Fuxian Lake constituted lineage B. Lineage C comprised a majority of specimens from the Haihe River, Taihu Lake, and Poyang Lake, and a minority of specimens from Khanka Lake and Fuxian Lake.

Conclusions This study indicates that native *M. nipponense* prawns in China originated from East China, subsequently spreading northward and westward into the inland regions along the Grand Canal and the Yangtze River system, forming distinct lineages. This proposed route improves our understanding of the geographic distribution and origin of *M. nipponense* in China.

Keywords *Macrobrachium nipponense*, Phylogenetic analysis, Mitogenomes, Genetic diversity

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Introduction

The oriental river prawn (*Macrobrachium nipponense*) is an economically important freshwater crustacean species found in East Asia (China, Japan, South Korea, Vietnam, and Myanmar) [1, 2]. It exhibits a high degree of environmental adaptability [3] and can migrate between fresh and brackish water (amphidromous). Unlike many other *Macrobrachium* species, *M. nipponense* does not require brackish water to reproduce [2, 3]. In China, *M. nipponense* is widely distributed in lakes and rivers. Due to the high intensity of human disturbances and the high connectivity among inland water systems, freshwater environments are especially susceptible to biological invasions [4–7]. The gene flow between estuarine populations is higher than the one between populations in inland freshwater populations [8]. A systematic geographical analysis of inland freshwater populations is essential to understand the biological and geological factors governing its distribution [8–10]. Mitochondria are cellular organelles crucial for energy synthesis and conversion in eukaryotes, whose genetic material was discovered in the early 1960 s [11]. Since then, researchers have discovered the mechanisms required for DNA replication, RNA transcription, and protein translation in mitochondria, including DNA polymerases, RNA polymerases, transfer RNAs, and ribosomal RNAs. Thus, mitochondria contain a relatively independent genetic transcription system [12, 13]. Generally, the mitochondrial genome of animals is a circular, double-stranded molecule between 10 and 39 kilobases (kb) in size, that is maternally inherited [14]. The mitochondrial DNA of shrimps is commonly a circular molecule between 15 and 20 kb in size, encoding 37 genes: 13 protein-coding genes (PCGs), 2 rRNAs, 22 tRNAs, and 1 control region (CR) [15, 16].

While mitochondrial DNA (mtDNA) is prone to producing artefactual relationships in deep phylogenies, it has been used effectively to analyze phylogenetic relationships among relatively closely related species [17–19]. In addition, the mitochondrial cytochrome C oxidase subunit I (*cox1*) gene is the foundation of the species barcoding system [20–22]. Provided that it exhibits intraspecies diversity, *cox1* can also be applied to study relationships among different populations [6, 23]. However, there is a growing number of reports of more than one mitochondrial genome type (mitotype) in the same organism, known as heteroplasmy [24], which can affect phylogenetic and haplotype network reconstruction, and cause erroneous description of relationships among populations [25]. Therefore, a multilocus approach is necessary to assess the reliability of molecular signal.

Most genetic studies of *M. nipponense* have focused on genetic variability at a regional scale [26–28]. Herein, we sampled 126 M. *nipponense* specimens from five geographical populations comprising four major freshwater lakes (Khanka Lake, Taihu Lake, Poyang Lake and Fuxian Lake) and one river (Haihe River) in China. We performed whole-genome resequencing of selected 100 samples and used the sequencing data to assemble mitogenomes for population analysis. The objective of the study was to investigate the genetic diversity and population structure of *M. nipponense* within and among these five populations using mitochondrial molecular markers (*cox1* and all 13 protein-coding genes—13 PCGs), a nuclear marker (*28S*) and SNPs. We also compared the mitochondrial genomes among five populations. Based on the analysis, we proposed the migration routes of *M. nipponense* in China. This study provides a direct insight into the origin and distribution of *M. nipponense* in China, which will help scientists better understand the evolutionary trajectory of this species.

Materials and methods

Sampling, DNA extraction, amplification, and sequencing

In this study, 126 oriental river prawns were collected from four freshwater lakes and one river (Fig. 1 and Supplementary Table S1). The localities spanned most of China, from north to south, and comprised three major river basins (Yellow, Yangtze, and Pearl Rivers). After collection, the samples were immediately frozen in liquid nitrogen or refrigerated and stored at −40 °C for DNA extraction. Total DNA extraction was carried out using an Ezup Column Animal Genomic DNA Purification Kit (Sangon, Shanghai, China) following the supplier's guidelines. We extracted the total DNA from the muscle tissue of *M. nipponense* (0.01–0.02 g) and verified its quality using 1.0% agarose gel electrophoresis. The mitochondrial *cox1* gene was amplified by PCR using the forward primer LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and reverse primer HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Table 1) in a reaction system containing 7 μl double distilled water, 1 μl template DNA (about 100 ng), 10 μl 2× Taq PCR MasterMix, and 1 μl primers (10 μM) [29]. The PCR reaction conditions were 95℃ pre-denaturation for 5 min; 30 cycles of denaturation at 95℃, annealing at 58℃, extension at 72℃ for 1 min; and final extension at 72℃ for 10 min.

Mitogenome sequencing and analyses

The complete mitogenome was sequenced for one specimen from each locality (i.e. five specimens in total) using next-generation sequencing following the Illumina's standard protocol. After quality checking of the genomic DNA, mechanical fragmentation was performed using ultrasound. The DNA fragments were

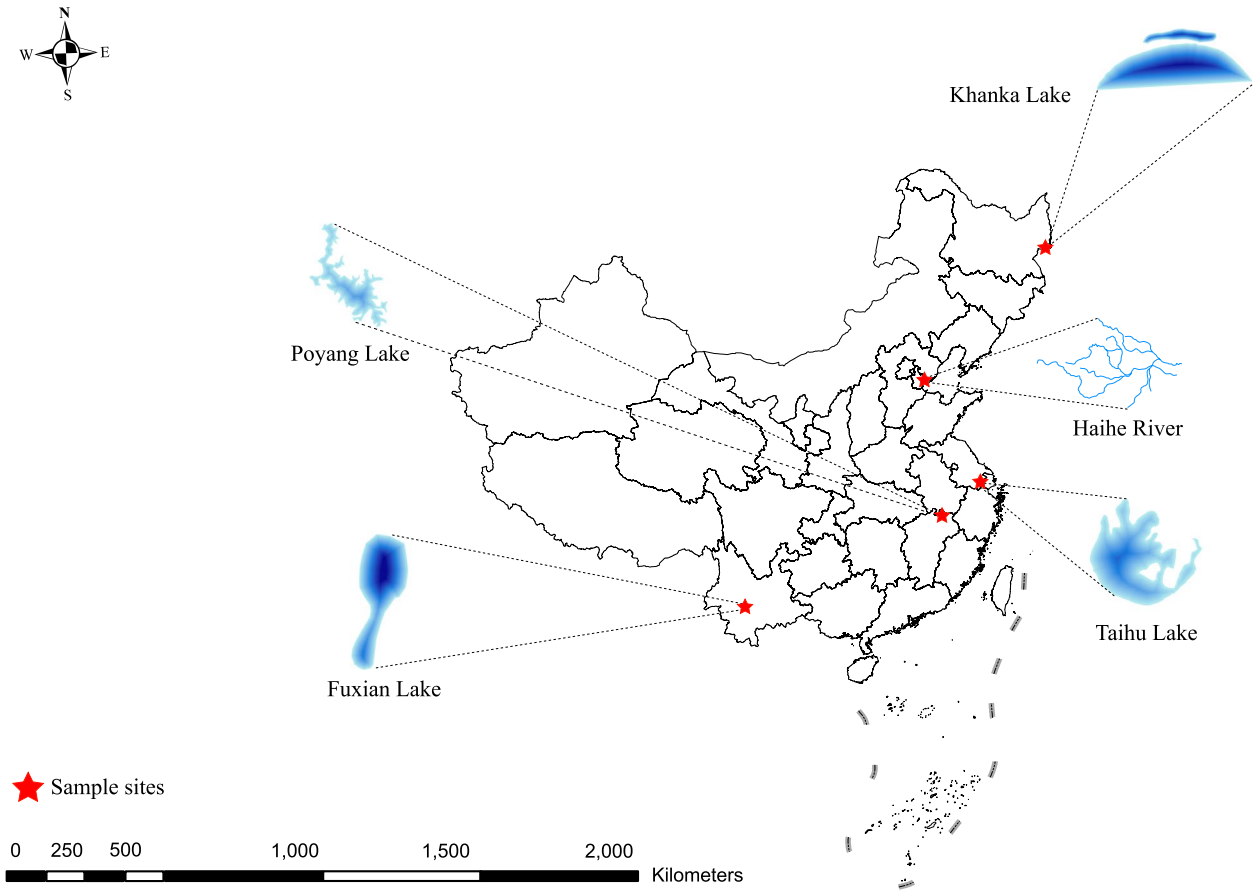

Fig. 1 A map showing the sampling sites, comprising four lakes and one river in China

Table 1 Genetic diversity parameters based on the cytochrome c oxidase subunit I gene for *M. nipponense*. N: The number of samples within the population, S: Number of polymorphic (segregating) sites, No. of mutations: Total number of mutation sites, InDel_Number: No. of InDel, h: No. of haplotypes, Hd: Haplotype diversity, Pi: Nucleotide diversity, K: Average of nucleotide differences

Population	Abbr	N	S	No. of mutations	InDel_ Number	h	Hd	π(Pi)	Pi(JC)	K
All		126	326	411	27	33	0.909 ± 0.012	0.03013 ± 0.00618	0.0335	18.079
Fuxian Lake	F	30	17	17	0	5	0.593 ± 0.082	0.00500 ± 0.00110	0.00504	3.067
Haihe River	H	27	100	111	9	9	0.749 ± 0.068	0.01811 ± 0.00739	0.01896	10.991
Poyang Lake	P	13	64	67	3	10	0.949 ± 0.051	0.01895 ± 0.00746	0.01952	11.577
Taihu Lake	T	28	66	69	9	13	0.894 ± 0.036	0.01186 ± 0.00427	0.01216	7.222
Khanka Lake	X	28	246	254	9	5	0.429 ± 0.108	0.03562 ± 0.02538	0.04762	21.728

purified, end-repaired, poly-A tailed at the 3'end and ligated with a sequencing adaptor. Agarose gel electrophoresis was used for fragment size selection, followed by PCR amplification to construct the sequencing library. The library was first subjected to quality inspection, followed by sequencing of the qualified library on the Illumina NovaSeq platform (Illumina Inc., San Diego, CA, USA) to generate 150 bp paired-end reads (PE150). The original data were filtered using fastp software (version 0.20.0, https://github.com/OpenGene/fastp).

Mitogenome assembly and gene annotation

The core module for assembly of the mitochondrial genome was SPAdes v3.10.1 (http://cab.spbu.ru/software/spades/) [30], which does not depend on the reference genome. The previously sequenced complete

mitochondrial genome sequence of *M. nipponense* [31] was used as the reference sequence (GenBank accession number: HQ830201.1, https://www.ncbi.nlm.nih.gov/nuccore/) for quality control after the assembly.

NCBI's ORF Finder and MITOS Web Server (http://mitos2.bioinf.uni-leipzig.de) [32] were used to annotate PCGs, selecting the invertebrate mitochondrial genetic code. MEGA 11.0 software [33] was employed to infer the codon usage pattern. The MITOS Web Server was employed to examine the tRNA cloverleaf secondary structures. The rRNA genes were annotated based on the locations of adjacent tRNA genes and compared with published mitogenome of a related species (*M. rosenbergii*) [34]. The CODEML program in the paml4 software package was used to analyze the ratios of nonsynonymous and synonymous replacement rates (Ka/Ks or dN/dS), which were estimated by a NG model using Ka_Ks calculator 2.0 [35, 36]. PhyloSuite (version 1.2.3) [37] was used to calculate the nucleotide content, base composition skews, and the Relative Synonymous Codon Usage (RSCU) values of the mitogenomes. The Organellar Genome DRAW tool was employed to construct a graphical map of the mitogenome [38, 39].

Genetic diversity and phylogeographic analysis

The number of variations of polymorphic (segregating) sites, insertion/deletion sites (InDel_Number), haplotypes (h), haplotype diversity (Hd), nucleotide diversity (Pi), Jukes-Cantor (JC) nucleic acid diversity (Pi JC) and nucleotide differences were calculated using DnaSP (software version: 6.0) [40]. The same software was further used to conduct neutrality tests, and calculate Tajima's *D* value, Fu and Li's *D** value, and Fu and Li's *F** value. The haplotypes between samples were also analyzed using DnaSP. Tajima's *D* [41] was used to check for deviations from neutrality, indicating whether population expansion had occurred in the past. Fu and Li's test [42] was also carried out to assess evidence for population expansion.

Arlequin software (version 3.5.2.2) [43] was used for molecular biological analysis of variance (Analysis of Molecular Variance Analysis, AMOVA) [44] to calculate the degree of freedom (df), sum of squares (SS), variance component (VC) and percentage of change (V%). Arlequin was also used to calculate the genetic differentiation index (*F*-statistic, F_{st}) among populations. In addition, population expansion was also investigated with mismatch analysis to examine the frequency distributions of nucleotide difference as a function of frequency by Arlequin.

Sample sequences were aligned using MAFFT v7.427 [45] (–auto mode), and then using DnaSP to load the alignment results and group the samples together to generate haplotypes. The haplotype network graph was constructed using the TCS Network [46] under the sub-function Network of PopART (Population Analysis with Reticulate Trees, version 1.7) [47]. Principal component analysis used cluster (version 3.0) software [48]. The genetic distance matrix (GD) was calculated using the Genetic sub-function under the Distance function of GenAlEx (version: 6.51b2) software [49]. The principal coordinates analysis (PCoA) scatter diagrams of principal coordinates 1 and 2 were drawn, and the preset populations were marked with different colors. The PCoA matrix corresponding to the first 10 principal components was subsequently derived.

Phylogenetic analyses

The haplotype sequences identified previously were used to construct a phylogenetic tree. Multi-sequence alignment was performed using MAFFT v7.427 (–auto mode). Under Bayesian information criterion, the optimal nucleotide substitution model was calculated by jModelTest v2.1.10 [50]. The haplotype Maximum likelihood (ML) phylogenetic tree was analyzed by using RAxML-NG v1.2.2 [51], the GTR model, and 1000 bootstrap repeats.

A whole genome resequencing was performed on 100 samples, the mitochondrial genome and nuclear genome datasets were constructed respectively for phylogenetic analysis [52]. Analysis of mitochondrial genome: Firstly, 13 PCGs were extracted from the assembled mitochondria (using SPAdes) and grouped by gene name. The 13 PCGs of 100 specimens were aligned using MAFFT v7.4.27, and then the aligned PCGs sequences were connected. Finally, ML phylogenetic tree was conducted using RAxML v8.2.10 [53], in combination with GTR-GAMMA model, and 1000 bootstrap replicates. For the nuclear genome, SNPs were identified using BWA v0.7.17 [54] to compare sample sequencing data with the reference genome (NCBI: PRJNA646023). BCFtools v1.9 [55] was used to extract SNP of *28S* from detected SNP, and SNP sites converted fasta format using vcf2phylip v2.0 [56]. FastTreeMP v2.1.11[57] was used to build a *28S* phylogenetic tree. The detected SNPs were filtered using VCFtools v0.1.16 [58] to obtain the trusted mutant site.

Phylogenetic trees based on the *cox1* datasets were reconstructed using the neighbor-joining method (NJ) [59] in MEGA. The bootstrap test (1000 replicates) [60] was used to assess the topological stability. The phylogenetic trees were visualized and annotated using the online tool iTOL [61]. The evolutionary distances were computed using the p-distance method [62] and given in the units of the number of base differences per site. Fewer than 50% alignment gaps, missing data, and ambiguous bases were allowed at any position; that is, all positions with less than 50% site coverage were eliminated [33].

Two mitochondrial markers (*cox1* and 13 PCGs) and a nuclear marker (*28S*) were used to infer the phylogenetic relationships among the oriental river prawn specimens. For the phylogenetic tree constructed using the *cox1* dataset, *Macrobrachium rosenbergii* [63] (GenBank accession number: PP337800) and *Macrobrachium maculatum* [27] (GenBank accession number: MW069513) were chosen as the outgroups. For the tree constructed using the 13 PCGs and *28S* datasets, *Macrobrachium hainanense* (GenBank accession number: PP747075) was obtained from GenBank and used as the outgroup.

Results

The composition, structure, and organization of the five mitogenomes

The complete mitochondrial genome (15,783 bp) of *M. nipponense* was a classical closed-loop molecule (Fig. 2A and Supplementary Fig. S1). Its nucleotide composition was biased towards A and T (A = 37.19% to 37.37%, T = 28.83 to 29.04%, C = 21.38 to 21.59%, G = 12.21% to 12.41%) (Table 2), and its structure was similar to that of other crustaceans [31, 64–66] (Table 3).

Protein-coding genes

With the exception of *cox1* (ACG start codon), all other PCGs in the five populations used typical ATN start codons: ATG for *atp6*, *cox2-3*, *nad1*, *nad3-5*, *nad4 l*, and *cob*; ATC for *atp8*, and *nad6*; and ATT for *nad2* (Table 3). Eight PCGs terminated with the standard stop codon TAA, but *nad1* and *nad4* terminated with TAG, and three genes (*cox1*, *cox2*, *cob*) terminated with an incomplete stop codon (T–). Figure 2B and Supplementary Table S2 summarize the RSCU analysis results. In addition to the start/stop codons, there were 3698 codons in the mitochondrial genome of *M. nipponense*, with the most common amino acids being Leu (CTR and TTR) (592), Ser (AGR and TCR) (357), and Ile (ATR) (296). Codons encoding Cys (TGR) (50) and Arg (CGR) (63) were uncommon.

rRNAs and tRNAs

The mitochondrial genome of all five specimens included 22 tRNA genes (63 bp to 69 bp). Among them, the H strand encoded 14 and the L strand encoded 8 (Table 3). Figure 3 shows the MITOS-predicted secondary structures of the 22 tRNAs. Except for the missing dihydrouridine loop structure (DHU) of *trnS1*, the other 21 tRNAs had the classic cloverleaf secondary structure, including the amino acid arm and loop, DHU arm and loop, anticodon arm and loop, and the thymidine-pseudouridine-cytidine (TΨC) arm or loop. Notably, we also found some mismatched base pairs and G-U pairing in secondary structures of most tRNAs (*trnA*, *trnC*, *trnF*, *trnG*, *trnH*, *trnI*, *trnP*, *trnQ*, *trnS1&S2*, *trnT*, *trnV*, *trnW*, *trnY*) (Fig. 3). In addition, the L strand contained two RNA genes: *rrnL* between trnL1 and *trnV* was 1282 bp, and *rrnS* between *trnV* and CR was 852 bp long (Fig. 2B and Fig. 3).

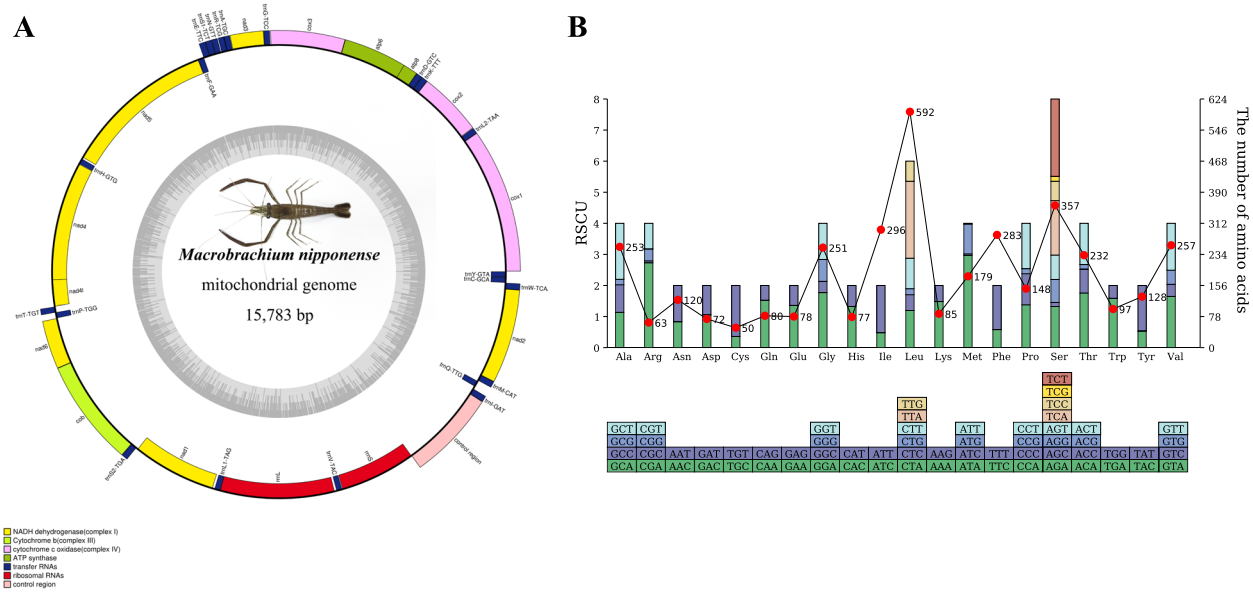

Fig. 2 Gene map of the *M. nipponense* mitogenome (**A**) and the statistic of relative synonymous codon usage (RSCU) in the mitogenome of *M. nipponense* (**B**). Three types of genes are represented in different colors: 13 protein-coding genes (purple, yellow, green, and light pink), rRNA genes (red), tRNA genes (blue). trnS1, trnS2, trnL1, and trnL2 denote codons tRNA-Ser (AGN), tRNA-Ser (UCN), tRNA-Leu (CUN), and tRNA-Leu (UUR), respectively. CR = control region

Table 2 Nucleotide composition (%) and skew value of *M.nipponense* mitogenomes in 5 populations. DNA base composition is shown as percentages. X, Khanka Lake; H, Haihe River; P, Poyang Lake; T, Taihu Lake; F, Fuxian Lake

Feature		Size(bp)	A%	T%	G%	C%	A + T%	G + C%	AT-skew	GC-skew
Mitogenome	X	15,783	37.20	29.04	12.38	21.38	66.24	33.76	0.123	−0.267
	H	15,783	37.19	28.83	12.39	21.59	66.02	33.98	0.127	−0.271
	P	15,784	37.19	28.95	12.39	21.47	66.14	33.86	0.125	−0.268
	T	15,783	37.19	28.90	12.41	21.50	66.09	33.91	0.125	−0.268
	F	15,774	37.37	28.97	12.21	21.46	66.33	33.67	0.127	−0.275
PCGs	X	11,128	27.63	36.84	16.75	18.77	64.48	35.52	−0.143	−0.057
	H	11,128	27.63	36.60	16.75	19.02	64.23	35.77	−0.140	−0.063
	P	11,128	27.63	36.75	16.72	18.89	64.39	35.61	−0.142	−0.061
	T	11,128	27.55	36.73	16.79	18.93	64.28	35.72	−0.143	−0.060
	F	11,128	27.69	36.85	16.72	18.74	64.54	35.46	−0.142	−0.057
tRNAs	X	1447	32.76	32.62	19.21	15.41	65.38	34.62	0.002	0.110
	H	1449	32.71	32.71	19.25	15.32	65.42	34.58	0.000	0.114
	P	1449	32.85	32.71	19.12	15.32	65.56	34.44	0.002	0.110
	T	1449	32.85	32.71	19.12	15.32	65.56	34.44	0.002	0.110
	F	1447	32.83	32.62	19.14	15.41	65.45	34.55	0.003	0.108
rRNAs	X	2134	30.32	39.13	20.24	10.31	69.45	30.55	−0.127	0.325
	H	2134	30.22	39.32	20.29	10.17	69.54	30.46	−0.131	0.332
	P	2134	29.94	39.36	20.62	10.07	69.31	30.69	−0.136	0.344
	T	2134	30.04	39.36	20.52	10.07	69.40	30.60	−0.134	0.342
	F	2135	30.30	39.34	20.28	10.07	69.65	30.35	−0.130	0.336
Dloop	X	949	42.89	37.20	8.54	11.38	80.08	19.92	0.071	−0.143
	H	950	42.11	37.16	9.37	11.37	79.26	20.74	0.062	−0.096
	P	950	42.53	37.47	9.05	10.95	80.00	20.00	0.063	−0.095
	T	950	42.53	37.47	9.05	10.95	80.00	20.00	0.063	−0.095
	F	946	43.13	37.53	8.56	10.78	80.66	19.34	0.069	−0.115

Selection analyses

To detect the impact of selection pressure, we calculated the ratio of nonsynonymous to synonymous substitutions (dN/dS), known as the ω value, for the *cox1* gene of 126 specimens from five different geographical populations. The dN/dS values inferred using the one-ratio model analysis was 0.970 (Table 4).

Population genetic diversity analysis

We identified 326 polymorphic sites and 411 mutation sites in the *cox1* gene sequences of 126 samples (Table 1). The five populations had an average haplotype diversity (h) of 0.909, ranging from 0.429 in the Khanka Lake population to 0.949 in the Poyang Lake population. The total average nucleotide diversity (π) across all populations was 0.030, ranging from 0.005 in Fuxian Lake to 0.036 in Khanka Lake. Thirty-three haplotypes were detected across all samples, with the Taihu Lake population being the most diverse (13), and the Khanka Lake and Fuxian Lake populations being the least diverse populations (5). The Khanka Lake population had the highest average nucleotide differences (K; 21.728). The haplotype network (Fig. 4A) and the ML tree (Fig. 4C) constructed based on 33 haplotypes exhibited congruent topological structures, with all haplotypes divided into three lineages (A, B and C). The lineage A comprised most of the Khanka Lake (Hap 20, 22, and 24) and Haihe River (Hap 33) haplotypes. The lineage B comprised Fuxian Lake (Hap 8, 25, 26, and 28) and Poyang Lake (Hap 8) populations. Lineage C was predominantly composed of specimens from the Haihe River, Poyang Lake and Taihu Lake (Hap 1–7, Hap 9–19 and Hap 29–32), in addition to a tiny number of specimens from Khanka Lake (Hap 21 and 23) and Fuxian Lake (Hap 27). Contrary to this, network graphs and trees obtained based on 101 haplotypes were not clearly divided into these three lineages (Fig. 4B and D). Two sub-lineages were found in lineage C in the network. The first sub-lineage included populations from Taihu Lake and Haihe River and a few individuals from Fuxian Lake and Poyang Lake. The second one comprised specimens from Poyang Lake and Taihu Lake, with a small number of samples from Haihe River and Khanka Lake. An interesting phenomenon was that individuals from lineages A and B were both found in

Table 3 Characteristics of *M.nipponense* mitogenomes in 5 populations. X, Khanka Lake; H, Haihe River; P, Poyang Lake; T, Taihu Lake; F, Fuxian Lake

Gene	Direction	Size(bp)					Start codon					Stop codon					Intergenic regions				
		X	H	P	T	F	X	H	P	T	F	X	H	P	T	F	X	H	P	T	F
cox1	+	1535	1535	1535	1535	1535	ACG	ACG	ACG	ACG	ACG	TA-	TA-	TA-	TA-	TA-	1	0	1	0	1
trnL2(taa)	+	64	64	64	64	64											0	0	0	0	0
cox2	+	685	685	685	685	685	ATG	ATG	ATG	ATG	ATG	T–	T–	T–	T–	T–	2	2	2	2	2
trnK(ttt)	+	68	68	68	68	68											0	0	0	0	0
trnD(gtc)	+	65	66	66	66	65											0	0	0	0	0
atp8	+	159	159	159	159	159	ATC	ATC	ATC	ATC ATC C	ATC ATC C	TAA	TAA	TAA	TAA	TAA	0	0	0	0	0
atp6	+	675	675	675	675	675	ATG	ATG	ATG	ATG	ATG	TAA	TAA	TAA	TAA	TAA	−7	−7	−7	−7	−7
cox3	+	789	789	789	789	789	ATG	ATG	ATG	ATG	ATG	TAA	TAA	TAA	TAA	TAA	−1	−1	−1	−1	−1
trnG(tcc)	+	65	65	65	65	65											6	6	6	6	6
nad3	+	354	354	354	354	354	ATG	ATG	ATG	ATG	ATG	TAA	TAA	TAA	TAA	TAA	0	0	0	0	0
trnA(tgc)	+	63	63	63	63	63											2	2	2	2	2
trnR(tcg)	+	63	63	63	63	63											−1	−1	−1	−1	−1
trnN(gtt)	+	65	65	65	65	65											6	6	6	6	6
trnS1(tct)	+	67	67	67	67	67											0	0	0	0	0
trnE(ttc)	+	69	69	69	69	69											0	0	0	0	0
trnF(gaa)	-	67	67	67	67	67											−2	−2	−2	−2	−2
nad5	-	1707	1707	1707	1707	1707	ATG	ATG	ATG	ATG	ATG	TAA	TAA	TAA	TAA	TAA	0	0	0	0	0
trnH(gtg)	-	64	64	64	64	64											18	18	18	18	18
nad4	-	1335	1335	1335	1335	1335	ATG	ATG	ATG	ATG	ATG	TAG	TAG	TAG	TAG	TAG	2	2	2	2	2
nad4 l	-	300	300	300	300	300	ATG	ATG	ATG	ATG	ATG	TAA	TAA	TAA	TAA	TAA	−7	−7	−7	−7	−7
trnT(tgt)	+	65	65	65	65	65											2	2	2	2	2
trnP(tgg)	-	65	65	65	65	65											−1	−1	−1	−1	−1
nad6	+	516	516	516	516	516	ATC	ATC	ATC	ATC	ATC	TAA	TAA	TAA	TAA	TAA	1	1	1	1	1
cob	+	1135	1135	1135	1135	1135	ATG	ATG	ATG	ATG	ATG	T–	T–	T–	T–	T–	−1	−1	−1	−1	−1
trnS2(tga)	+	69	69	69	69	69											0	0	0	0	0
nad1	-	942	942	942	942	942	ATG	ATG	ATG	ATG	ATG	TAG	TAG	TAG	TAG	TAG	19	19	19	19	19
trnL1(tag)	-	65	65	65	65	65											23	23	23	23	23
rrnL	-	1282	1282	1282	1282	1282											0	0	0	0	0
trnV(tac)	-	66	66	66	66	66											21	21	21	21	21
rrnS	-	852	852	852	852	853											4	4	4	4	4
D-loop	+	949	950	950	950	946											0	0	0	0	−3
trnI(gat)	+	67	67	67	67	67											0	0	0	0	0
trnQ(ttg)	-	68	68	68	68	68											31	29	29	29	27
trnM(cat)	+	68	68	68	68	68											8	8	8	8	8

Table 3 (continued)

Gene	Direction	Size(bp)					Start codon					Stop codon					Intergenic regions				
		X	H	P	T	F	X	H	P	T	F	X	H	P	T	F	X	H	P	T	F
nad2	+	996	996	996	996	996	ATT	ATT	ATT	ATT	ATT	TAA	TAA	TAA	TAA	TAA	0	0	0	0	0
trnW(*tca*)	+	69	69	69	69	69											−2	−2	−2	−2	−2
trnC(*gca*)	-	62	62	62	62	62											1	1	1	1	1
trnY(*gta*)	-	63	64	64	64	63											0	0	0	0	0

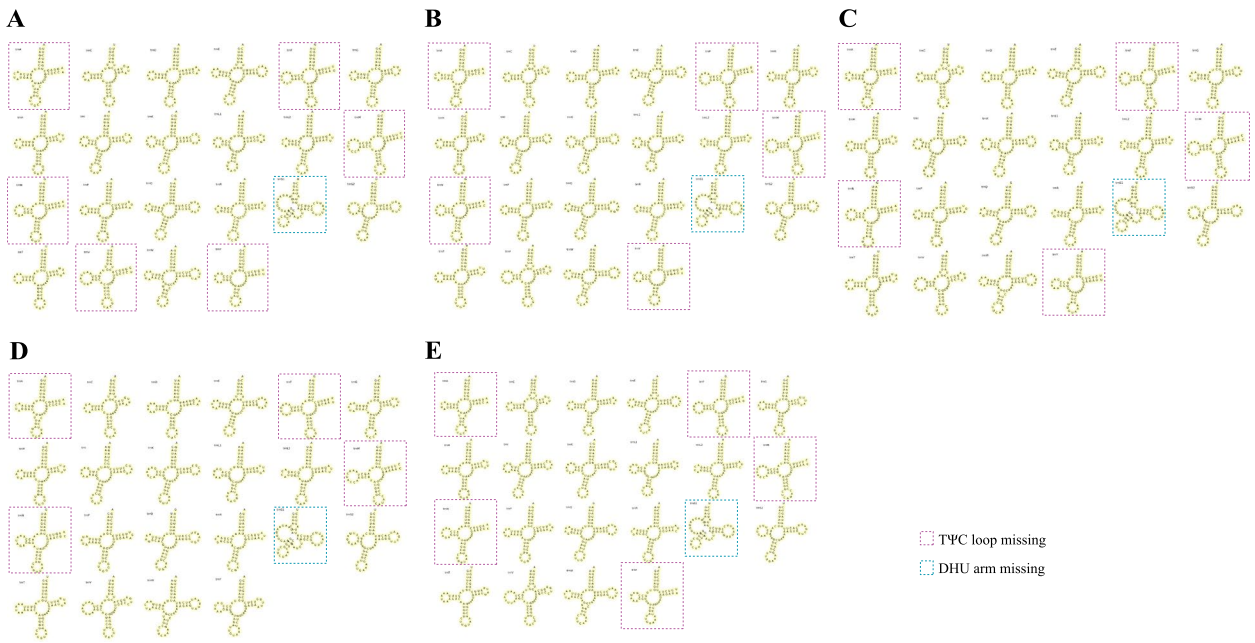

Fig. 3 Secondary structures of 22 tRNAs from *M. nipponense*. The populations from A to E are Khanka Lake, Haihe River, Taihu Lake, Poyang Lake, and Fuxian Lake respectively

the Haihe River (Hap 33) and Khanka Lake populations (Hap 21 and Hap 23). In addition, both A and C lineages were found in the Poyang Lake (Hap 8) population (Fig. 4 and Supplementary Table S3). In the entire haplotype network, Hap 1 was the most common haplotype: it was identified in 68 individuals from three populations. It is worth noting that lineages B and C were genetically relatively close, i.e. separated by fewer mutational steps than lineage A in relation to B/C.

Phylogenetic analysis

The NJ phylogenetic analysis of mitochondrial markers (*cox1* and 13 PCGs) and ML phylogenetic of a nuclear marker (*28S*) also revealed three major clades, corresponding to the three lineages. In the mitochondrial phylogenetic trees, most of the remaining specimens from the Khanka Lake population, along with one Haihe River specimen, formed the clade A. The clade B was comprised mostly of the Fuxian Lake population, along with one specimen from the Poyang Lake and one from the Khanka Lake populations. Finally, all of the Taihu Lake specimens and most of the Haihe River and Poyang Lake specimens formed a large and diverse clade C. The clade also comprised one Fuxian Lake and several Khanka Lake specimens (Fig. 5A and B). In the nuclear marker tree, we observed that the Haihe River population and the Khanka Lake population formed sister clades (Fig. 5C). Considering uncertainties encountered in the present study, the SNPs were further to understand the phylogenetic relationships among the different populations of this species (Fig. 5D). We observed that the Fuxian Lake populations were distinct, and the specimens from the Khanka Lake were nested inside, rendering it paraphyletic (Fig. 5C and D). The reconstruction of the evolutionary history of *M. nipponense* populations indicated that they spread gradually from East China to other lakes (Fig. 6).

Structure of the populations and their demographics

AMOVA showed that most variation in the *cox1* sequences was detected within populations (5.285), so the within-population variation (52.79%) was greater than the between-population variation (47.21%) (Supplementary Table S4). The results of gene flow analysis showed a significant gene flow among the Haihe River, Poyang Lake, and Taihu Lake populations (Supplementary Table S5). The population genetic differentiation index (F_{st}) ranged from 0.009 to 0.685 (Supplementary Table S6). The genetic distance within the populations was evaluated based on the results of the phylogenetic tree analysis. The Fuxian Lake population had the smallest genetic distance (0.005), while the Khanka Lake population had the largest (0.036) (Supplementary Table S5). Furthermore, the Haihe River, Poyang Lake, and Taihu

Table 4 Analysis on pressure selection of *M. nipponense* from five river systems

#ID	One-ratio(lnL)	dN	dS	ω(dN/dS)
cox1	−4102.87	5.235	5.400	0.970

Lake populations showed high similarity regarding genetic distances and phylogenetic analysis (Fig. 4 and Supplementary Fig. S2).

We used DnaSP 6.0 software to perform neutrality test analysis on the five populations (Table 5). A significant Tajima's *D* value (− 2.530, $P < 0.01$) was found in most populations, apart from the Fuxian Lake population. The data indicate that, except for the F population, the populations have undergone expansion and directional selection during their evolutionary history; while the Fuxian Lake population is more consistent with a neutral evolution model (Table 5 and Supplementary Fig. S3).

Discussion

The mitochondrial genome features

The mitochondrial genomes of all five *M. nipponense* specimens exhibited a structure consistent with other decapods [67–70]. All five mitochondrial genomes had a typical crustacean skew pattern, with a positive AT-skew and a negative CG-skew, which is considered the ancestral state for crustacean mitogenomes [71, 72]. This is associated with the replication mechanism, which in turn depends on the direction of the control region (CR), located between the *rrnS* (-strand) and *trnI* (+ strand) genes in the ancestral arthropod gene order (AAGO) [18, 73, 74], and contains the origins of replication (O_H and O_L) [75, 76]. We observed the presence of incomplete stop codons in the PCGs, which is common in animal mitochondrial genes [77, 78]. *Macrobrachium nipponense* exhibits a canonical tRNA secondary structure, consistent with reports regarding the mitochondrial genomes of other decapods [16, 34,

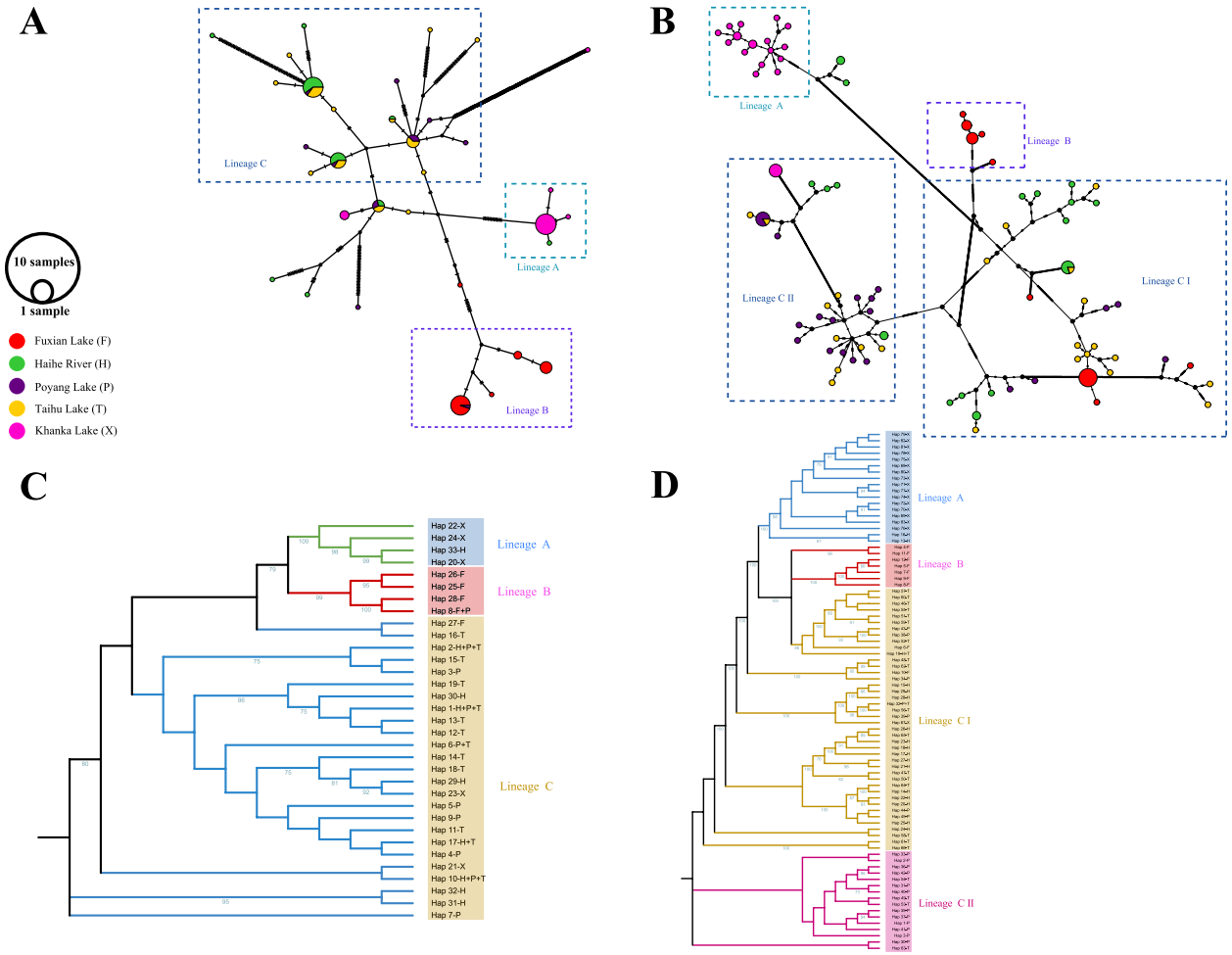

Fig. 4 Haplotype networks and maximum likelihood (ML) phylogenetic trees inferred using two haplotype sequences datasets. In haplotype networks, the size of the circle is proportional to the frequency of a particular haplotype. Each small line on the line that connects two circles represents a mutational step and black dots represent hypothetical missing intermediates

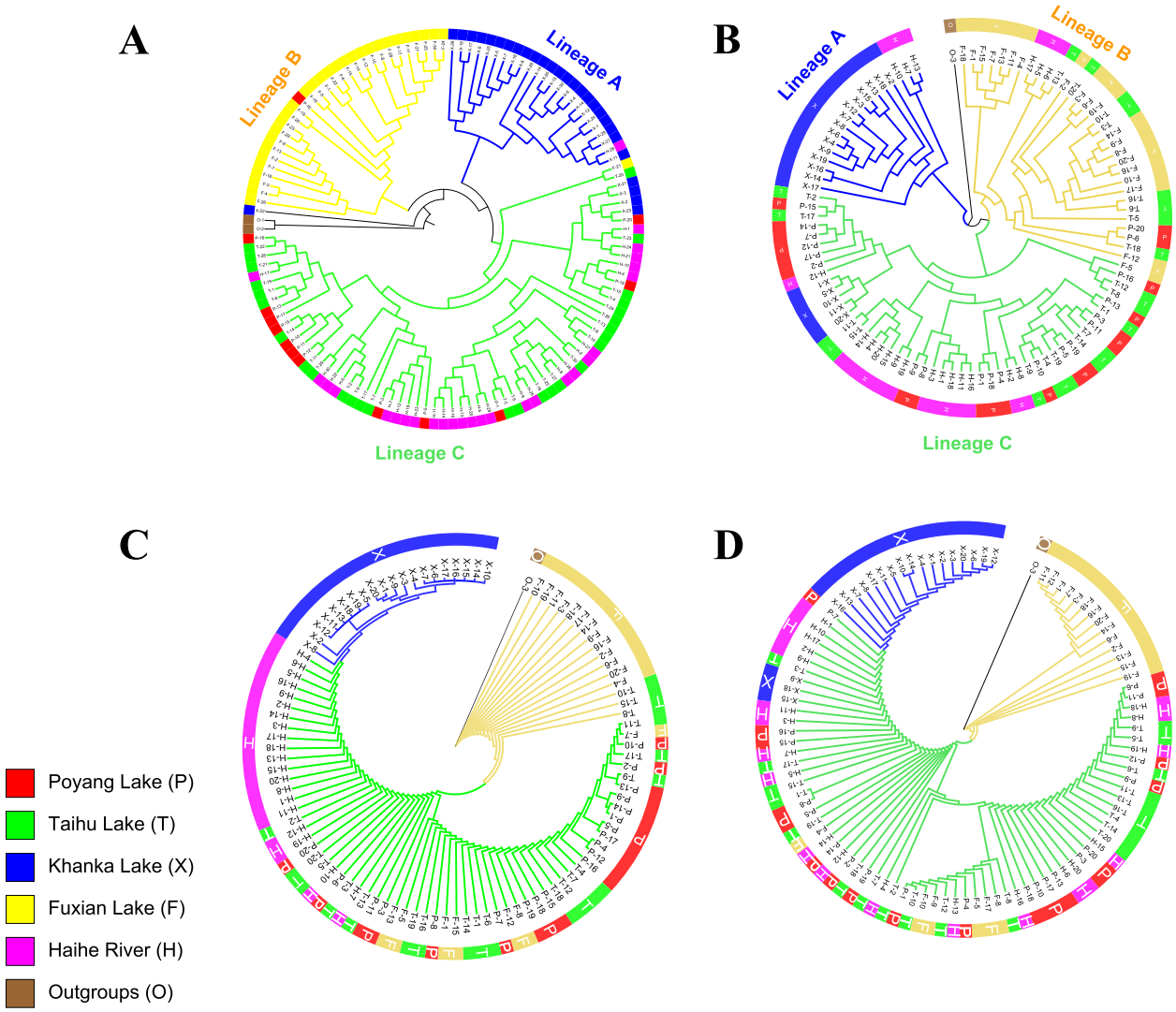

Fig. 5 Phylogenetic trees inferred using the neighbor-joining method for mitochondrial markers (*cox1* and 13 PCGs, A and B respectively) and maximum likelihood method for the nuclear marker (*28S*, C) and SNPs (D). Outgroups: O-1 is *M. maculatum*, O-2 is *M. rosenbergii*, O-3 is *M. hainanense*

65, 79, 80]. Most identified tRNAs could be folded into the standard cloverleaf structure. However, *trnA*, *trnF*, *trnM*, *trnN*, and *trnY* genes did not have the TΨC loop in all populations, with the following exceptions: *trnV* was also missing this element in the Khanka Lake population, and *trnY* was not missing it in the Poyang Lake population (Fig. 3). The missing TΨC arm in *trnV* does not appear to be common among the crustaceans, but it was also observed in *Ligia oceanica* and *Ichthyoxenos japonensis* [81, 82]. In addition, we found that *trnD*, *trnP*, and *trnR* possess a simplified TΨC loop comprising only three nucleotides, which was also found in other arthropods, such as *Macrophthalmus pacificus* [83], *Synalpheus kensleyi* [84], and Perlodidae [85]. It is worth noting that the lack of a DHU arm in *trnS1* is considered a typical feature of metazoan mitogenomes [86]. The secondary structure of lacking DHU arm is considered to be the structural compensation mechanism among tRNA arms [87]. We observed that the most common non-Watson–Crick base pair is G-U (or U-G) wobble base pair. According to previous studies, these non-Watson–Crick pairings do not affect the functioning of tRNAs because these minor mismatches are corrected later via tRNA modifications [88, 89].

Nonsynonymous and synonymous substitutions

The calculation of nonsynonymous (dN) and synonymous (dS) substitutions plays a crucial role in understanding

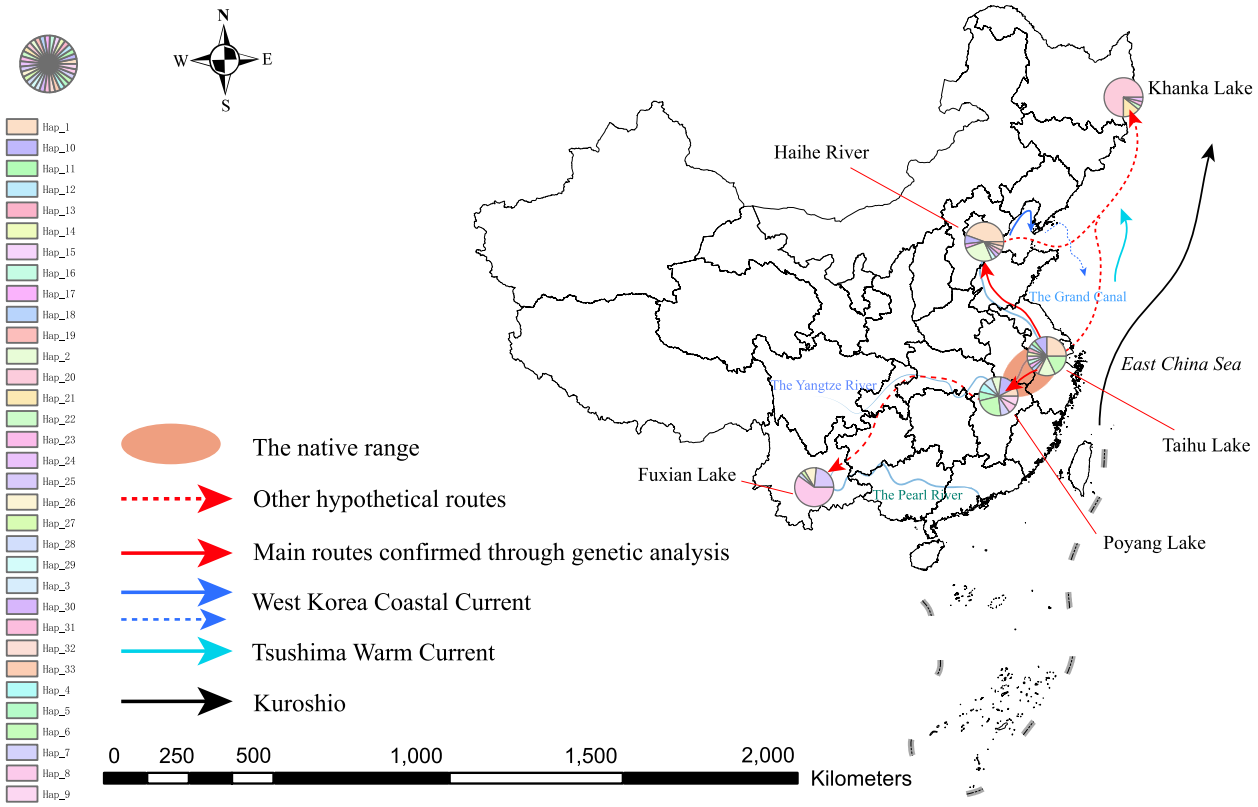

Fig. 6 The dispersal routes of *M. nipponense* in China, based on mitochondrial DNA

Table 5 Neutral test of *M.nipponense* in 5 lake systems based on *cox1* gene

Population	Abbr	Tajima's *D* statistic (p value)	Fu and Li's *D* test statistic (p value)	Fu and Li's *F* test statistic (p value)
All		−2.530(***)	−9.281(**)	−7.401(**)
Fuxian Lake	F	−0.976(Not significant)	−2.694(*)	−2.526(Not significant)
Haihe River	H	−2.409(**)	−4.096(**)	−4.183(**)
Poyang Lake	P	−2.095(**)	−2.674(**)	−2.883(**)
Taihu Lake	T	−2.265(**)	−4.187(**)	−4.199(**)
Khanka Lake	X	−2.617(***)	−4.765(**)	−4.791(**)

the evolutionary dynamics of PCGs across closely related species [90, 91]. Purifying selection is generally considered the dominant force in the molecular evolution of mitochondrial genomes [92–94]. However, several species of aquatic animals appear to exhibit signs of positive selection, reflecting their adaptations to environmental changes, energy metabolism, and physiological functions [95–98]. In this study, the dN/dS value of the *cox1* gene was almost 1, which indicates strongly relaxed purifying selection pressure [72]. A previous study has shown that loss of locomotory capacity can lead to a relaxation of purifying selection pressures in crustacean mitogenomes [99], and *M. nipponense* does have a relatively weak swimming ability [100], so this might be considered as the underlying cause for this finding.

Population relationships

As a result of the long-term accumulation of mutations, populations with ancestral genotypes often retain higher nucleotide and haplotype diversity [101, 102]. In this study, we propose two competing hypotheses. First hypothesis: *Macrobrachium nipponense* originated in the area where the Taihu Bay and the estuary intersect. With the accumulation of silt, the Yangtze River Delta alluvial plain was formed, from which *M. nipponense* spread to the inland areas, and the population stabilized

in Poyang Lake over a long period. Second hypothesis: *Macrobrachium nipponense* originated from a large lake formed by the Taihu and Poyang Lakes, or their geographical distance was short in the past, and geological activities separated the lakes into their current state over time. However, this assumption should be interpreted with caution, for there is no substantial evidence (such as fossils) to prove the correlation between geological activities and *M. nipponense*. The invasion of freshwater populations usually occurs in estuarine areas [103], so it was possible for *M. nipponense* to disperse from estuaries under the influence of some potential factors (such as ocean currents).

The scattered distribution of rivers can form a geographical barrier for freshwater biotas, and limit the opportunities for gene flow between different river populations [104]. However, this study found no significant genetic differentiation between the Haihe River, Taihu Lake, and Poyang Lake populations. There was a significant differentiation between the estuarine population of Haihe River and the inland populations of Khanka Lake and Fuxian Lake. Previous research indicated that *M. nipponense* is widely distributed in major rivers and reservoirs in Chinese Taiwan, and these populations have dispersed through specific routes, exhibiting high genetic connectivity [26]. However, the migration range of oriental river prawn in the estuary areas is limited due to its weak swimming ability and strong territorial awareness [105–107]. Therefore, the critical factor causing variation between populations is the diffusion of the early life stage larvae, and the influence of ocean currents is the main driving force for species diffusion [108]. A study reported that the zoea larvae of *M. nipponense* metamorphosed into the juvenile stage after 28 days [26, 109], which provides a sufficiently long period for the larvae to migrate and ensures frequent gene exchanges among different estuarine populations [26].

We thus believe that the origin of *M. nipponense* was in the East China, followed by a northward range extension into the Haihe estuary in the Yellow Sea, promoted by the Grand Canal and the coastal current [110]. In addition, the Khanka Lake population of *M. nipponense* showed the highest nucleotide diversity and the lowest haplotype diversity compared to other populations. This phenomenon may occur in long-term stable populations. The haplotypes are restricted because of historical events (such as natural selection), whereas nucleotide variation accumulates over time. It might also reflect historical gene flow events, where a few haplotypes were introduced between different populations, but these haplotypes accumulated more nucleotide variations over time [111]. However, in the Haihe River, Poyang Lake, and Taihu Lake populations, the results are the opposite, generally exhibiting high haplotype diversity. This indicates that these population have rapidly expanded, resulting in many new haplotypes in a short period of time, with insufficient time for the accumulation of nucleotide diversity. Another possibility is that the population has recovered after a bottleneck, which strongly reduced the nucleotide variation, with new haplotypes emerging from a few survivors. *Procambarus clarkii* also exhibited the highest haplotype diversity in its native area [112], which agrees with our findings.

Even though the phylogeographic analysis in the present study corroborated the separation of the *M. nipponense* populations into three lineages, the heteroplasmy may have affected the haplotype analyses (i.e. haplotypes shared between lineages and incongruent clusters), as observed in previous studies of *Macrobrachium amazonicum* [113, 114]. The few samples from Poyang Lake and Taihu Lake that contradicted the expected pattern may represent examples of microheteroplasmy that diverged from the predominant haplotypes observed in these localities. Furthermore, as described above, Taihu Lake and Poyang Lake are regions where numerous waters meet, that were related genetically to the coastal populations (Haihe River) was found in Lineage C, which may reflect the high level of heteroplasmy in Taihu Lake and Poyang Lake populations, or indicate that this region represents an ancient contact zone among populations.

Dynamic dispersal of river prawn in China

We present the hypothetical migration routes of *M. nipponense* in China (Fig. 6). Global warming can drive species to extend their distribution towards polar regions and migrate to higher altitudes. Conversely, cooling climate may drive species towards lower latitudes [115]. In addition, water temperature can affect the development of the gonads and the metabolic levels of sex hormones in oriental river prawns, with suitable water temperatures promoting gonadal development and activating sex hormones [116, 117]. In this study, 28 samples came from the cold temperate zone (Khanka Lake) and 30 from the subtropical zone (Fuxian Lake). During the north-to-south migration of *M. nipponense*, the temperature also gradually increased. This temperature gradient also created selection pressures that caused a change in population distributions (Table 5 and Supplementary Table S7). Similar results of temperature adaptability affecting geographical distribution have been found in other crustaceans and fishes [118–120]. However, the outcomes of such selective pressures might have been influenced by complex climatic or geological historical events. It is worth noting that haplotypes of both Khanka Lake and Fuxian Lake populations showed relative independence compared to other populations, providing a new

perspective on the geographical distribution of *M. nipponense*. During the last glacial period (the Holocene), the global sea level dropped, and Khanka Lake underwent several minor regression and transgression events [121]. During this period, many primitive forest and plankton communities appeared on the Khanka Lake plain, forming a unique wetland system and providing a rich food source and suitable environment for *M. nipponense*. In addition, high Hd and low π genetic characteristics were found in the Fuxian Lake geographical population. Similar genetic characteristics were reported in another fish population from Fuxian Lake [122]. It is possible that during the Quaternary Ice Age, the temperature dropped sharply, causing the biological populations of Fuxian Lake to shrink dramatically, resulting in a considerable loss of low-frequency alleles. However, upon entering the Pliocene epoch, with a warming climate, many plankton species began to reproduce in the lake. Under the conditions of abundant food, *M. nipponense* reproduced rapidly, and the population number was restored. DNA variation usually takes much longer to restore than the number of haplotypes [123–125], thus resulting in a"high Hd low π"evolutionary feature observed herein.

Conclusions

The complete mitochondrial genomes of *M. nipponense* specimens from five different populations were sequenced, and their tRNA secondary structures were predicted. Four phylogenetic trees and two haplotype networks, constructed using SNPs, two mitochondrial markers (*cox1* and 13 PCGs) and a nuclear marker (*28S*), revealed that the sampled *M. nipponense* specimens could be divided into three major clades. The genetic diversity of different geographical populations was determined by comparing their haplotype diversity, nucleotide diversity, and average nucleotide differences. We observed a highly similar genetic structure across populations in the Middle and Lower Reaches of the Yangtze River Plain in China. Using phylogenetic analysis, we attempted to reconstruct past migration routes of *M. nipponense*. The origin of this species was in East China, from where it gradually spread westward along the Yangtze River Basin. This study demonstrates that *M. nipponense* has extensive environmental adaptability, encompassing both inland lakes and coastal river environments in China. This contributes to addressing questions concerning the phylogenetic evolution and geographic migration of *M. nipponense*. This evolutionary and genetic analysis of *M. nipponense* offers an insight into the evolutionary strategies employed by different *Macrobrachium* species in adaptation to different environments, as well as an insight into predicting its geographic distribution in the future in response to the changing climate.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11692-w.

Supplementary Material 1

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Authors' contributions
PH Luo: Writing-review & editing, Software, Project administration, Investigation, Data curation, Conceptualization. YT Jin: Software, Methodology, Investigation, Formal analysis, Data curation. T Zhao: Investigation, Formal analysis, Data curation. C Bian: Software, Methodology, Investigation, Formal analysis, Data curation. ZM Lv: Methodology, Investigation, Formal analysis, Data curation. N Zhou: Software, Data curation, Conceptualization. JG Qin: Software, Data curation. SM Sun: Writing-review & editing, Methodology, Investigation, Data curation.

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Data availability
All raw data results can be obtained from the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1106180, accessed in May 2024).

Declarations

Ethics approval and consent to participate
All animal care and experimental procedures were approved by the Animal Experiment Committee of Shanghai Ocean University, China (approval no. SHOU-DW-2024–007). The experiments in this study comply with the current laws of China.

Consent for publication
Not applicable.

Competing Interests
The authors declare no competing interests.

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