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RESEARCH ARTICLE

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Salinity appears to be the main factor shaping spatial *COI* diversity of Corbicula lineages within the Chinese Yangtze River Basin

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Abstract

Aim: To date, few studies have examined the phylogenetics of Corbicula clams in their native range and the environmental parameters influencing their distribution, although this could provide great insights into the biological adaptation and invasion dynamics of Corbicula clams. We sought to identify the genetic lineages of native Corbicula clams and elucidate the environmental factors shaping the distributions of identified lineages.

Location: China, mainly the Yangtze River Basin.

Methods: The alignment comprised 558 *COI* sequences including samples from China and 222 *COI* sequences from published studies. This dataset was used to generate phylogenetic trees and compare population diversity. We used dbRDA method to assess the relationship between these *COI* data and environmental factors measured to identify the important factors affecting Corbicula's distribution.

Results: The *COI* phylogenetic tree delineated the monophyly of 3 major *COI* clades and 77 distinct *COI* haplotypes in the Yangtze River Basin. The invasive lineage C/S (FW17) was not identified in our sampling in China, while invasive lineages A/R (FW5 = Hap6), B (FW1 = Hap17) and Rlc (FW4 = Hap43) were identified, abundant and widely distributed in the middle and lower reaches of Yangtze River. Focusing on populations from Yangtze River Basin, both the *COI* haplotype and nucleotide diversity in the lakes along the Yangtze River increased with longitude, except for the river mouth population. The AMOVA tests showed significant differentiation between the middle and lower reaches of the Yangtze River and among populations. The dbRDA results suggested that the parameter chloride explained most of the spatial *COI* haplotype distribution variation in the Yangtze River Basin, with the three invasive lineages tolerating broad fluctuations of salinity (chloride levels ranging from 4 to 60 mg/L).

Conclusions: The Corbicula *COI* haplotypes found within the Yangtze River Basin had distinct distribution preferences, with the invasive androgenetic lineages being the

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most abundant and widely distributed. Genetic diversity was higher in this native region than in invaded areas in Europe and America, while it decreased with increasing distance from the river mouth. Salinity appeared to be the main environmental factor shaping the *COI* haplotype distribution of Corbicula lineages within their native range.

KEYWORDS
Asian clam, evolutionary adaptation, genetic diversity, haplotype distribution, lineages, oceanic origin

1 | INTRODUCTION

Biological invasions have been unambiguously shown to be among the major causes of global biodiversity decline (Courchamp et al., 2017; Diagne et al., 2021). Genus Corbicula, also known as Corbicula complex, is considered one of the most ecologically and economically costly freshwater invaders (Haubrock et al., 2022; Pigneur, Falisse, et al., 2014; Strayer, 2010). The original distribution of Corbicula (Bivalvia, Cyrenidae; formerly Corbiculidae) seems to be confined to Asia, Africa and Australia (Araujo et al., 1993); since the mid-20th century, they have established a global distribution that spans all continents except the Antarctic (Bespalaya et al., 2018; Crespo et al., 2015). In general, Corbicula clams are regarded as high-impact freshwater invaders due to their ability to detrimentally alter community and ecosystem dynamics, through nutrient cycling and energy flow, phytoplankton depletion and competition for resources and substrate modification (Pigneur, Falisse, et al., 2014; Sousa et al., 2014). Its diverse reproductive strategies (androgenetic hermaphrodite, sexual dioecism, cross-fertilization and self-fertilization) is another issue of interest in their invasion biology (Pigneur et al., 2012). Although the rapid expansion of Corbicula across its invasive range is mostly linked to anthropogenic-mediated dispersal (Coughlan et al., 2017), the invasive Corbicula lineages all appear to be hermaphrodites reproducing through androgenesis, and one individual is sufficient to establish a new population (reviewed in Pigneur et al., 2012). These Cyrenidae are therefore considered a potential model taxon for the general study of rapid bivalve radiations into inland environments (Graf, 2013).

Few studies have attempted to assess the phylogenetics and phylogeography of sympatric Corbicula clams within their native habitats, in Asia, Africa or Australia. As a result, the evolutionary processes that have shaped historical radiation and adaptation in Corbicula, as well as their contemporary interactions with environmental preferences, remain unclear. One of the dominant drivers of species distribution is habitat (McDowell et al., 2014). Because of their global distribution and high invasive efficiency (Crespo et al., 2015), the Corbicula's habitat preference for environmental factors such as temperature, turbidity and salinity has been studied (Avelar et al., 2014; McMahon, 1979; Müller & Baur, 2011; Sousa et al., 2006). Low temperature (<2°C), high temperature (>25°C) and high turbidity (>150 nephelometric turbidity units, NTU) could cause a decline in their survival rates (Avelar et al., 2014; McMahon, 1979; Müller & Baur, 2011), and it was also reported that changes in salinity could influence their abundance and biomass (Sousa et al., 2006). The consequences of these environmental factors could directly shape their distribution and colonization (Ferreira-Rodríguez et al., 2017), but it remains unclear whether different effects exist between species (lineages) within the genus.

Despite an almost global distribution, the taxonomy of this genus is poorly understood, primarily due to the extraordinary range of morphological variation and unique reproductive modes, including both sexual dioecy and androgenetic hermaphrodite species (Gomes et al., 2016; Komaru et al., 2013; Peñarrubia et al., 2017; Pigneur et al., 2011). In recent years, with the development of sequencing methods, molecular markers have been employed to solve taxonomical difficulties (e.g. Bespalaya et al., 2018; Haponski & Ó Foighil, 2019; Vastrade et al., 2022). Two Corbicula mitochondrial lineages are now considered to be widely distributed within Asian freshwater environments using *COI*, forms A/R and B (Park & Kim, 2003; Pigneur, Etoundi, et al., 2014; Wang et al., 2014). However, discrepancies remain between genetic and morphological identifications (Lee et al., 2005; Siripattrawan et al., 2000), as well as between nuclear and mitochondrial markers (Hedtke et al., 2008; Lee et al., 2005; Peñarrubia et al., 2017; Pfenninger et al., 2002; Vastrade et al., 2022). The taxonomical identification of Corbicula also remains difficult because of their androgenetic mode of reproduction and polyploidy.

Despite the limitations of relying on a single molecular marker, the widely used *COI* marker has shown to be reliable for Corbicula, and was used in this study. Hence, in this study, we sampled Corbicula clams from the middle and lower Yangtze River Basin and produced mitochondrial *COI* sequences, downloaded available *COI* sequences of Corbicula clams from the NCBI database and identified the mitochondrial lineages present in the native and non-native regions. The spatial distribution of these lineages was further investigated in the populations of the middle and lower Yangtze River, and environmental indicators were used to investigate the drivers influencing their distributions. We tested the hypothesis of whether salinity was the main factor influencing the distribution of Corbicula in the Yangtze River Basin. These results will improve our understanding of the adaptive mechanisms of their invasion and provide information for biodiversity conservation in invaded areas to control their colonization.

2 | METHODS

2.1 | Sample collection

Corbicula specimens were collected with hand net or dredge from seven freshwater lakes and the Yangtze River mouth (YzM, $n = 60$). Sampled lakes included five of the largest freshwater lakes in China: Chao Lake (ChL, $n = 4$), Dongting Lake (DoL, $n = 126$), Hongze Lake (HzL, $n = 20$), Poyang Lake (PoL, $n = 19$) and Tai Lake (TaL, $n = 30$), as well as two other lakes situated within the Yangtze River Basin: Datong Lake (DaL, $n = 55$) and Dianshan Lake (DsL, $n = 22$) (see Figure S1 and S2a–g for the location of sampled lakes and estuary and sampling localities within the lakes, respectively, and Table S1 for the sampling time). Lakes Datong (DaL), Dongting (DoL) and Poyang (PoL) are located in the middle reaches of the Yangtze River Basin, while the other four lakes are situated in the lower reaches. Currently, only Dongting Lake and Poyang Lake are directly connected with the Yangtze River, the other lakes are indirectly connected through a network of tributary rivers, except Hongze Lake. Located in the lower reaches of the Huai River Basin, Hongze Lake is directly connected to the Yangtze River by the Beijing-Hangzhou Grand Canal. Specimens were washed on-site using a 0.5 mm mesh sieve with clean water and were immediately preserved in ethanol until DNA extraction.

2.2 | DNA extraction and sequencing

Genomic DNA was extracted from each specimen's mantle tissue using the TIANamp tissue DNA kit (Tiangen Biotech Co., Ltd.). A 710-base-pair (bp) fragment of the mitochondrial *COI* gene was then amplified using the universal primers LCO1490/HCO2198 (Folmer et al., 1994). PCRs were conducted in a final reaction volume of 25 μl consisting of 100 ng template DNA, 25 mM $MgCl_2$, 1 × PCR buffer, 2.5 mM each dNTP, 10 μM each of primers, 2.5 U of Taq DNA polymerase (Tiangen) and ddH_2O. The reactions were conducted in an Applied Biosystems 2720 thermal cycler (Singapore), with an initial denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension step of 72°C for 5 min. Following this, 3 μl of PCR products were electrophoresed and visualized on 1% ethidium bromide (EB)-stained agarose gels to confirm single products. PCR products were purified using a TIANgel Midi Purification Kit (Tiangen Biotech Co., Ltd.). Products were sequenced by Sangon Biotechnology (Shanghai) Co., Ltd. (China) using Applied Biosystems™ 3730xl DNA Analyzer, and the sequences were subjected to quality control independently in Geneious (R11, Biomatters Ltd.), BioEdit (Hall, 1999) and Sequencher 4.1.4 (Gene Codes Corporation).

2.3 | Phylogenetic analysis and haplotype network

As morphological traits cannot be relied upon for taxonomical identifications in Corbicula due to phenotypic plasticity, *COI* sequences were used here to identify lineages within this complex. To examine the phylogenetic relationship of Corbicula clams, other *COI* sequences from specimens collected throughout China were obtained from the NCBI database and the literature, including a few reference sequences (Table 1). However, as the sequences obtained from GenBank varied in length, only sequences with a length >550 bp were considered in our analyses. All sequences from Chinese Corbicula individuals were aligned using the plugin ClustalW Alignment (Thompson et al., 1994) in Geneious (Gap Open Cost = 100 and Gap Extend Cost = 10: R11, Biomatters Ltd.). Moreover, the *COI* sequences of the four identified invasive lineages in Europe and America (Hedtke et al., 2008; Park & Kim, 2003; Pigneur et al., 2011; Pigneur, Etoundi, et al., 2014; Siripattrawan et al., 2000; Vastrade et al., 2022), being FW5 (form A/R, NCBI ID: AF196268) (Siripattrawan et al., 2000), FW1 (form B, AF196269) (Siripattrawan et al., 2000), FW4 (form Rlc, AF269096) (Renard et al., 2000) and FW17 (form C/S, AF269095) (Renard et al., 2000), were added to the alignment and phylogenetic reconstruction to compare native Chinese and invasive Corbicula specimens using *COI*. Furthermore, *Corbicula spp.* not previously documented in China were also included in the alignment and *COI* phylogeny: *C. sandai* (AF196272), *C. japonica* (AF196271) (Siripattrawan et al., 2000), *C. loehensis* (AY275666), *C. matannensis* (AY275663), *C. moltkiana* (AY275657), *C. possoensis* (AY275661) (Glaubrecht et al., 2003) and *Batissa violacea* (Outgroup, DQ837727) (Glaubrecht et al., 2006).

All sequences in the alignment were trimmed to the same length. *COI* haplotypes were generated using DnaSP v 6.12.03 (Rozas et al., 2017), while the haplotype network was produced using the median-joining network method with POPART 1.7 (Leigh & Bryant, 2015). Phylogenetic relationships were studied between the *COI* haplotypes by constructing a maximum-likelihood (ML) phylogenetic tree. The best nucleotide substitution model was selected based on the lowest AICc value by ModelFinder (Kalyaanamoorthy et al., 2017) and was used to build the maximum-likelihood phylogenetic tree using IQ-TREE (Nguyen et al., 2015).

Furthermore, to visualize the spatial genetic variation and the abundance of Corbicula clams residing within the middle and lower reaches of the Yangtze River Basin, haplotypes from DnaSP were mapped along the Yangtze River using ArcGIS 10.2 (ESRI Inc).

2.4 | Genetic diversity

This study focused on Corbicula from the middle and lower reaches of the Yangtze River, and only populations associated with the Yangtze River were retained for the following analyses, including ChL, DaL, DsL, DoL, HzL, PoL, TaL, and YzM. *COI* genetic diversity was evaluated for all the Corbicula specimens recovered from the Yangtze River Basin (Table 3), the analyses computed the number of haplotypes (*h*), the haplotypic diversity (*hd*) and the nucleotide diversity (π) in DnaSP.

Considering the sampling size, which is uneven here, the ChL ($n = 4$) population was further excluded in the structure analysis to

TABLE 1 Sources of Corbicula specimens and sequences used in the present study

Sites	Code	Specimens (collected in this study)	Sequences (downloaded from NCBI)	Total
(a) Samples and sequences from China				
Chao Lake	ChL	4	0	4
Dongting Lake	DoL	126	16[a]	142
Dianshan Lake	DsL	22	0	22
Datong Lake	DaL	55	0	55
Fujiang River	FuR	0	30[c]	30
Hong Kong	HoK	0	3[d]	3
Hongze Lake	HzL	20	78[e]	98
Poyang Lake	PoL	19	14[a]	33
Qingshan Lake	QiL	0	13[a]	13
Tai Lake	TaL	30	36[c]	66
Taiwan	TaW	0	28[a]	28
Yalu River	YaR	0	1[f]	1
Yangtze River Mouth	YzM	60	0	60
Yunan	YuN	0	3[g]	3
Total		336	222	558
(b) Sequences for references				
Corbicula loehensis	AY275666	0	1	4[h]
C. matannensis	AY275663	0	1	
C. moltkiana	AY275657	0	1	
C. possoensis	AY275661	0	1	
C. japonica	AF196271	0	1	2[i]
C. sandai	AF196272	0	1	
FW1 Form B	AF196269	0	1	3[a]
FW4 Form Rlc	AF269096	0	1	
FW5 Form A/R	AF196268	0	1	
FW17 Form C/S	AF269095	0	1	1[j]
Batissa violacea	DQ837727	0	1	1[h]

Note: Specimens sampled and sequences retrieved from GenBank from China, published data are marked with subscript letters, references: [a]Park & Kim, 2003; [b]Wang et al., 2014; [c]Iida et al., 2012; [d]Pfenninger et al., 2002; [e]Li et al., 2015; [f]Yamada et al., 2014; [g]Pigneur, Etoundi, et al., 2014; [h]Glaubrecht et al., 2003; [i]Siripattrawan et al., 2000; [j]Renard et al., 2000.Sequences available in GenBank for reference, citations and accession numbers are provided.

avoid bias. The Yangtze River is usually divided into middle (=Middle) and lower (=Lower) reaches by the Hukou (which is the boundary for middle and lower reaches of Yangtze River), so this study assigned DaL, DoL and PoL into Middle Yangtze River Basin and the other lakes (DsL, HzL, TaL and YzM) into Lower Yangtze River Basin. An analysis of molecular variance (AMOVA) was performed to determine the amount of genetic variability using *F-statistics* among geographic populations and among clades. The significance of the covariance components associated with the different possible levels of genetic structure was tested by 10,000 permutations. AMOVA was performed in Arlequin v3.5.2.2 (Excoffier & Lischer, 2010). For the uneven sampling size, we performed another AMOVA analysis considering the populations with sample size >30 for all populations and populations of clade II (see Table S2a,b) and compared the results with the first analysis. Both analyses yielded similar results and therefore the first analysis was retained within this study (Table 4).

2.5 | Environmental parameter measurements and statistical analyses

To assess the extent to which Corbicula haplotypes and environmental parameters influence the spatial distribution of Corbicula *COI* lineages, 18 environmental parameters were measured, including temperature (Temp), transparency (Trans), pH, dissolved oxygen (DO), conductivity (Cond), Chlorophyll-a (Chla), turbidity (Turb), chloride (Cl), calcium (Ca), magnesium (Mg), potassium (K), sodium

(Na), total phosphorus (TP), total nitrogen (TN), nitrate (NO^{3-}-N), nitrite (NO_2^{2-}-N), ammonium (NH_4^+-N), orthophosphate (PO_4^{3-}-P) and permanganate index (COD_{Mn}), for Chao Lake, Datong Lake, Dianshan Lake, Dongting Lake, Hongze Lake, Poyang Lake and Tai Lake. These 18 environmental parameters for each lake were collected at different times, and the specific sampling details are shown in Table S1 and Figure S2, and finally, the average of all time points was used for subsequent analysis (Table 2). Temp, pH, DO, Cond and Turb were measured using an YSI 6600 V2-4 Multi-Parameter Water Quality Sonde, and Trans was measured in the field using Secchi disk. Chloride (Cl) was determined by ion chromatography, and Ca, Mg, K and Na were measured using ICP-AES. Samples for the determination of NH_4^+-N, NO^{3-}-N, PO_4^{3-}-P and Chla concentration were filtered using Whitman GF/F filters. Chla was determined spectrophotometrically after extraction in 90% hot ethanol. TP, TN, NO^{3-}-N, NO_2^{2-}-N, NH_4^+-N, PO_4^{3-}-P and COD_{Mn} were measured in the laboratory based on standard methods (Rice et al., 2012). The measurements and analyses of these environmental parameters were completed by the Nanjing Institute of Geography and Lakes, Chinese Academy of Sciences. The data are displayed in Table 2.

The relationship between our *COI* haplotypes and environmental data was tested using distance-based multivariate linear model analysis (DistLM) in the statistical software Primer-e PERMANOVA+ (Anderson et al., 2008). The relationships between *COI* haplotype frequencies and environmental parameters were initially examined by analysing each predictor separately (marginal tests), and then sequentially using an adjusted R^2 selection procedure while avoiding collinearity between environmental data. Matrices in DistLM analyses were built using Bray–Curtis resemblance matrices of haplotypic abundances and Euclidean distance resemblance matrices of normalized 18 environmental parameters. The *p*-values for individual predictor variables were obtained using 9999 permutations (Leduc et al., 2012). Distance-based redundancy analysis (dbRDA) plots were also generated using PERMANOVA+ to visualize the results once the best DistLM model of each population was obtained. Specimens recovered from unmentioned lakes were excluded from this analysis as no corresponding environmental data were available for the locations.

3 | RESULTS

A total of 336 specimens were collected from seven lakes, as well as the Yangtze River mouth. All specimens were successfully amplified and sequenced to obtain *COI* barcodes for each individual (Table 1). After combined with sequences extracted from databases and previous studies presented in the literature (Table 1), the final alignment comprised 558 *COI* sequences of 526 bp including samples from China and 222 mitochondrial DNA samples documented by other studies from Corbicula sampled in other regions of the world. The 526 bp *COI* sequences comprised only 77 haplotypes.

3.1 | Haplotype phylogenetics

The *COI* phylogenetic tree obtained through ML delineated the monophyly of three major clades with relatively high support values: clades I, II and III (Figure 1). There was also additional *Corbicula spp.* that clustered outside these three major clades based on the examined sequences, such as *C. possoensis*, *C. loehensis*, *C. matannensis* and *C. moltkiana* from Malaysia and *C. sandai* from Japan (Figure 1). The *COI* haplotype FW17 retrieved in invasive lineage C/S also clustered outside the three clades (Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014) and was not identified in our sampling in China, neither in other Chinese populations retrieved from other studies. Clade III occupied a basal position relative to clades I and II and clustered together 35 *COI* haplotypes from our study with the brackish water species *C. japonica* from Japan (Figure 1). Clade I included haplotype FW5 of invasive lineage A/R (corresponding to Hap 6 in our study) that is widely distributed in Europe and America (Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014) and five additional *COI* haplotypes from China (Figure 1). FW5 (Hap6) was the only haplotype of clade I detected in our sampled lakes along the middle and lower Yangtze River (Figure 2). Clade II included not only 36 *COI* haplotypes from China but also FW1 of invasive Corbicula lineage B and FW4 of invasive lineage Rlc.

3.2 | Population diversity in middle and lower Yangtze River Basin

Among all 77 *COI* haplotypes we retrieved, the most common haplotype in our Chinese studied populations was *COI* FW5 (Hap6) of invasive lineage A/R (frequency: 126/558, Figure 1) and *COI* FW1 (Hap17) of invasive lineage B (frequency: 120/558, Figure 1). The most common *COI* haplotype in the estuarine clade III was Hap24 (frequency: 46/558, Figure 1).

Focusing on populations from Yangtze River Basin, *COI* haplotypes from the present study belonging to clade I were only observed in the middle reaches of the Yangtze River Basin and a few specimens in Hongze Lake (HzL), including only FW5 (Figure 2, a and b). Haplotypes from clade III were restricted to the lower reaches of the Yangtze River Basin and the estuary, comprising the estuarine lineages (Figure 2, e and f). In contrast, haplotypes from clade II were found across the whole middle and lower Yangtze River Basin, except in the Yangtze River mouth (YzM) (Figure 2, c and d) and included invasive Corbicula lineages B and Rlc, corresponding here to haplotypes Hap 17 (FW1) and Hap 43 (FW4) respectively. *COI* haplotypes FW1 and FW4 exhibited only one nucleotide difference and were both distributed widely in the middle and lower reaches of the Yangtze River (Figure 2, c and d). Haplotypes from clade II were found in sympatry with individuals from the other two clades (I and III). No lakes in the Yangtze River Basin were found to be inhabited by clams with *COI* haplotypes belonging to all three clades, except HzL, which does not belong to the basin but is connected by the Beijing-Hangzhou Grand Canal.

TABLE 2 Environmental parameters measured in each lake: The average value ± standard deviation of all measurements taken during several sampling months is presented

Environmental Parameter	DaL	DoL	PoL	ChL	HzL	TaL	DsL
Temperature/Temp (°C)	27.6 ±0.16	28.3 ±0.36	25.1 ±0.80	29.9 ±0.14	28.2 ±0.28	28.4 ±0.63	26.4 ±0.21
Transparency/Trans (m)	0.31 ±0.05	0.46 ±0.16	0.41 ±0.09	0.29 ±0.07	0.21 ±0.04	0.38 ±0.10	0.38 ±0.03
pH	8.2 ±0.48	8.1 ±0.08	7.4 ±0.13	9.3 ±0.30	9.1 ±0.09	8.5 ±0.08	8.6 ±0.08
Dissolved oxygen/DO (mg/L)	7.6 ±1.58	7.5 ±0.10	7.3 ±0.41	7.4 ±0.40	6.7 ±0.57	7.3 ±0.43	7.1 ±0.12
Conductivity/Cond (μs/cm)	319.13 ±12.76	228.14 ±45.95	97.03 ±16.4	258.08 ±35.11	479.75 ±73.36	476.43 ±102.86	672.00 ±27.27
Chlorophyll-a/Chla (μg/L)	9.18 ±8.45	1.70 ±0.77	3.42 ±0.66	4.98 ±0.91	5.33 ±3.06	21.75 ±13.25	3.22 ±0.69
Turbidity/Turb (mg/L)	29.50 ±5.79	40.30 ±22.25	39.87 ±22.59	46.40 ±14.54	93.79 ±38.42	31.92 ±12.16	23.56 ±2.90
Chloride/Cl (mg/L)	14.80 ±0.22	5.31 ±2.11	4.58 ±1.17	21.96 ±4.07	42.91 ±8.83	43.79 ±15.49	63.68 ±16.22
Calcium/Ca (mg/L)	29.92 ±3.14	27.49 ±4.93	10.10 ±1.99	21.58 ±2.44	29.11 ±2.41	28.26 ±2.01	43.45 ±3.91
Magnesium/Mg (mg/L)	13.20 ±0.22	6.79 ±1.30	1.89 ±0.25	5.84 ±0.63	12.98 ±2.05	7.52 ±1.34	11.58 ±0.60
Potassium/K (mg/L)	5.81 ±0.17	2.37 ±1.77	1.95 ±0.30	3.63 ±0.50	4.69 ±0.82	5.18 ±0.65	9.02 ±0.17
Sodium/Na (mg/L)	9.38 ±0.42	5.60 ±2.06	4.33 ±0.58	17.99 ±3.42	49.72 ±17.79	39.40 ±13.60	82.94 ±7.86
Total phosphorus/TP (mg/L)	0.06 ±0.004	0.03 ±0.004	0.07 ±0.02	0.12 ±0.02	0.09 ±0.02	0.08 ±0.03	0.21 ±0.04
Total nitrogen/TN (mg/L)	0.50 ±0.06	0.65 ±0.04	1.36 ±0.19	1.58 ±0.28	1.86 ±0.49	2.28 ±0.87	2.23 ±0.30
Nitrate/NO_3^--N(mg/L)	0.25 ±0.19	1.70 ±0.82	0.87 ±0.15	0.13 ±0.17	1.23 ±0.61	0.63 ±0.26	0.64 ±0.18
Nitrite/NO_2^{2-}-N (mg/L)	0.04 ±0.04	0.01 ±0.01	0.03 ±0.01	0.01 ±0.02	0.01 ±0.01	0.05 ±0.07	0.15 ±0.07
Ammonium/NH_4^+-N (mg/L)	0.42 ±0.19	0.99 ±1.08	0.21 ±0.06	0.16 ±0.03	0.11 ±0.05	0.54 ±0.20	0.42 ±0.29
Orthophosphate/ PO_4^{3-}-P (μg/L)	129.35 ±10.98	43.61 ±22.07	14.92 ±5.25	4.64 ±0.88	28.76 ±13.53	8.79 ±3.97	157.50 ±40.43
Permanganate index/ COD_{Mn} (mg/L)	6.04 ±0.41	2.93 ±0.38	3.73 ±1.55	6.20 ±2.47	4.13 ±0.88	3.87 ±0.74	4.17 ±0.18

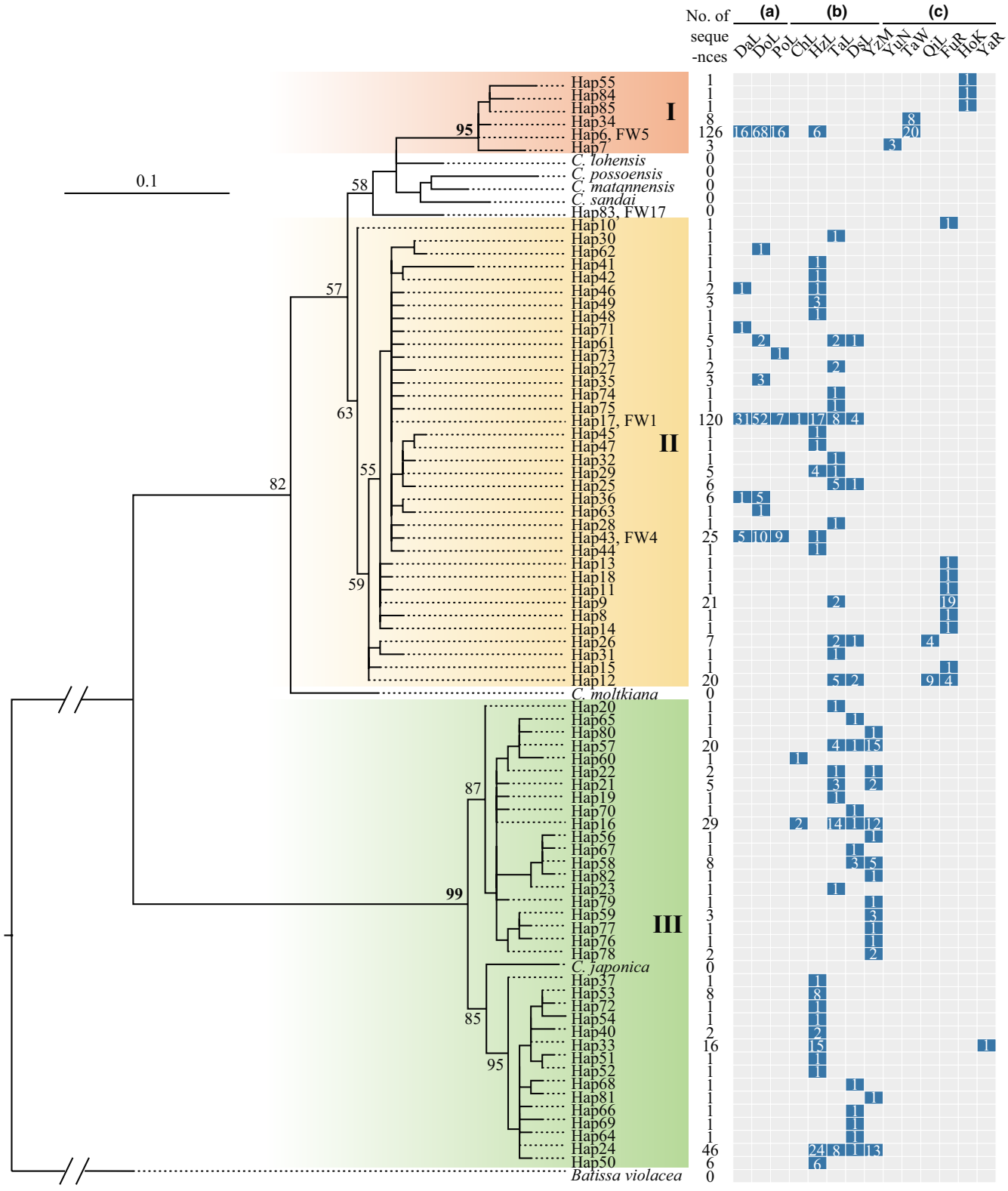

FIGURE 1 Phylogenetic tree inferred from ML analyses and haplotype geographic distribution of *COI* haplotypes for Corbicula lineages. Bootstrap values (%) are indicated at each node. Each haplotype shared by different populations and its number of sequences displayed in the right panel; a and b being, respectively, middle and lower reaches of Yangtze River, and c representing populations outside of Yangtze River that we did not sample (QiL without specific location).

Among different Corbicula populations, Dianshan Lake (DsL) displayed the highest *COI* haplotype (0.957) and nucleotide diversity (0.0464) in the Yangtze River Basin (Table 3). The lowest *COI* haplotype diversity (0.599) and the lowest nucleotide diversity (0.0080) were observed, respectively, in the Datong Lake (DaL) and the Yangtze River mouth (YzM), located at both extremities

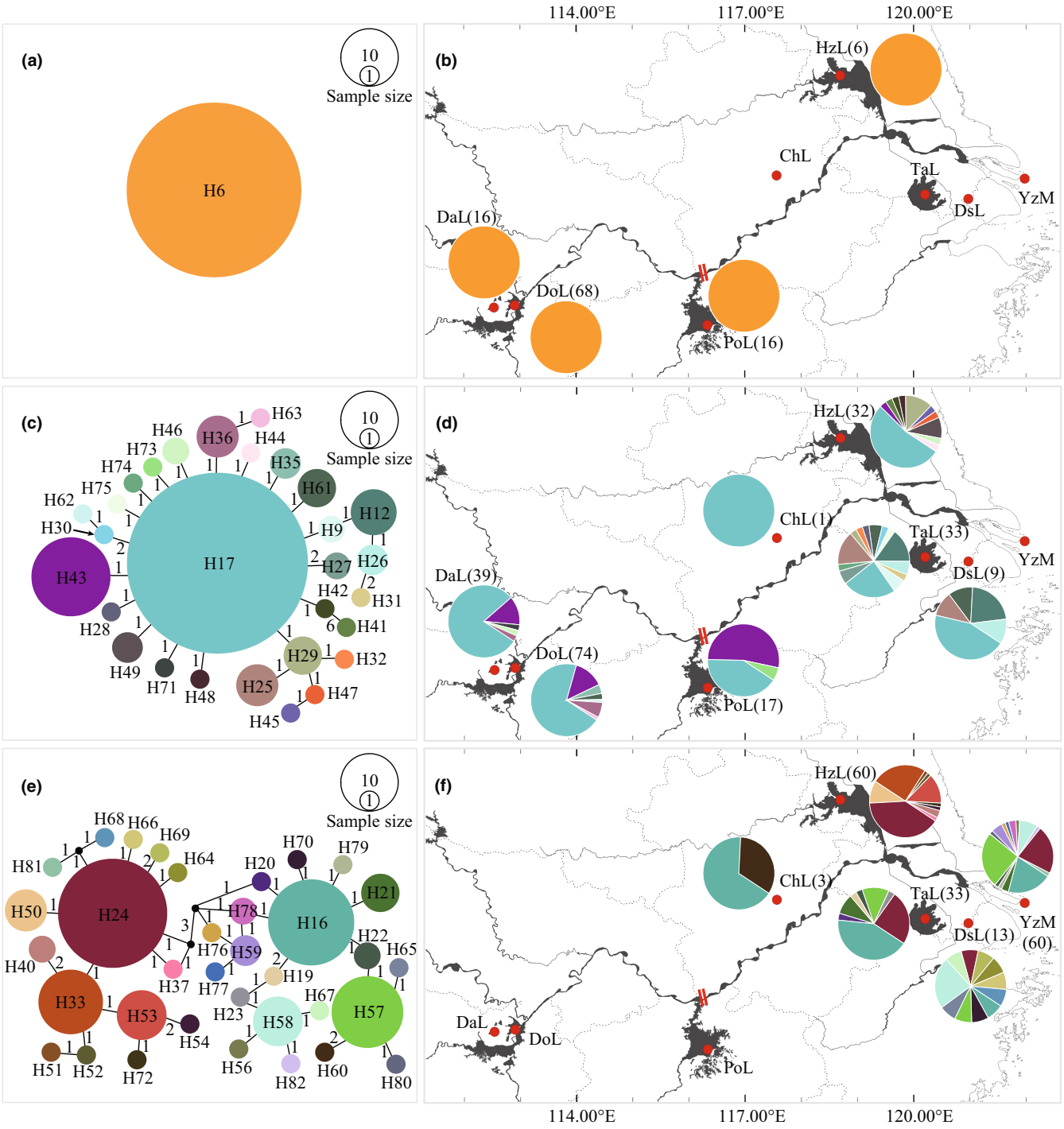

FIGURE 2 *COI* haplotype distributions of clades I (a and b), II (c and d) and III (e and f) in each Corbicula population along the middle and lower Yangtze River with populations outside of Yangtze River excluded. (a), (c) and (e) were haplotype networks per clade, (b), (d) and (f) were maps of part of the Yangtze River with the populations/sampling sites pointed with the haplotype distribution and abundance. In (a), (c) and (e), "H," standing for "Hap," with numbers corresponding to haplotype names in Figure 1; circle size being proportional to the number of sequences per haplotype, black dots and numbers next to solid lines representing undetected or hypothetical haplotypes and mutational steps between haplotypes respectively. In (b), (d) and (f), numbers in parentheses represent the number of sequences. The double solid red lines represent Hukou, which is the boundary for middle and lower reaches of Yangtze River.

of our sampling region. Both the haplotype and nucleotide diversity in the lakes along the Yangtze River increased with longitude, except for the river mouth population (Table 3), reflecting an increase in *COI* genetic diversity in the downstream direction of the river course (i.e. flowing from west to east) (Figure 2). The AMOVA tests showed significant differentiation between the middle and lower reaches of the Yangtze River and among populations using the *COI* data ($p < .001$, Tables 4a and S2a). For Corbicula in the

TABLE 3 Genetic diversity of Corbicula among different geographic populations

Population	*N*	*h*	*Hd*	π	Longitude
DaL	55	6	0.599 ±0.052	0.0117 ±0.0013	112.54° E
DoL	142	8	0.634 ±0.025	0.0139 ±0.0002	112.93° E
PoL	33	4	0.665 ±0.050	0.0146 ±0.0006	116.27° E
ChL	4	3	0.833 ±0.222	0.0440 ±0.0210	117.57° E
HzL	98	22	0.877 ±0.018	0.0423 ±0.0022	118.72° E
TaL	66	22	0.916 ±0.018	0.0441 ±0.0009	120.16° E
DsL	22	16	0.957 ±0.029	0.0464 ±0.0033	120.97° E
YzM	60	15	0.851 ±0.024	0.0080 ±0.0005	121.96° E

Abbreviations: *h*, number of haplotypes; *hd*, haplotype diversity; *N*, sample size; π, nucleotide diversity.

TABLE 4 AMOVA analysis of different geographic populations in the Yangtze Basin

Source of variation	Df	Sum of squares	Components	Percentage
(a) All geographic populations				
Between region	1	2003.496	7.816	50.46***
Among populations	6	458.213	1.392	8.98***
Within populations	472	2964.699	6.281	40.55*
Total	479	5426.408	15.489	
(b) Geographic populations in clade II				
Between region	1	6.587	0.04693	7.69
Among populations	5	7.514	0.04096	6.71*
Within populations	198	103.431	0.52238	85.60***
Total	204	117.532	0.61027	
(c) Geographic populations in clade III				
Among populations	4	135.321	1.07106	34.95***
Within populations	164	326.880	1.99317	65.05
Total	168	462.201	3.06423	

Note: Populations of the AMOVA analysis: DaL, DoL, PoL, ChL, HzL, TaL, DsL and YzM and the regions included in the analysis: middle reaches and lower reaches of Yangtze River. Only one haplotype (Hap6) was detected in the collected geographic populations in clade I, and the AMOVA test was therefore not applicable. Samples in clade III were only found in the lower region of the Yangtze River, and the hierarchical level of the region was not applicable.*$p < 0.05$; ***$p < 0.001$.

Yangtze Basin, all samples in clade I belonged to the same *COI* Hap 6, displaying no differentiation. In clade II, including haplotypes from almost the entire middle and lower basin regions, 85.60% of the genetic *COI* differentiation came from within populations ($p < .001$, Table 4b). In clade III, restricted to the lower basin region, a significant differentiation was observed among populations in the region ($p < 0.001$, Table 4c).

3.3 | Environmental factors shaping the haplotype's spatial distribution

Following the removal of the 12 variables that did not explain the distribution (Temp, Trans, pH, DO, Ca, K, Na, TN, NO_3^--N, NO_2^{2-}-N, NH_4^+-N and COD_{Mn}), marginal tests resulting from DistLM analysis of *COI* population genetic variation at the regional scale showed that the 98.72% of the spatial *COI* haplotype distribution variation could be explained by chloride (Cl, 50.93%), turbidity (Turb, 23.00%), total phosphorus (TP, 17.37%) and magnesium (Mg, 7.42%) (Figure 3). Chloride, whose concentrations were shown to increase with sodium and conductivity, was positively correlated with the *COI* distribution of Corbicula from Tai Lake (TaL) and Dianshan Lake (DsL) and negatively with Datong Lake (DaL), Dongting Lake (DoL) and Poyang Lake (PoL). The composition of *COI* haplotypes in Hongze Lake (HzL) was possibly impacted by the high turbidity. Accordingly, the *COI* variation observed between geographic populations and the significant *p*-value of chloride suggested that salinity likely affected Corbicula's distribution and dispersal along the river.

4 | DISCUSSION

4.1 | Three *COI* corbicula clades detected in China

Based on the phylogenetic tree and haplotype networks constructed using the mitochondrial *COI* marker, only three major Corbicula clades were grouped within the Yangtze River Basin (Figures 1 and 2). Previous studies documented seven freshwater Corbicula species

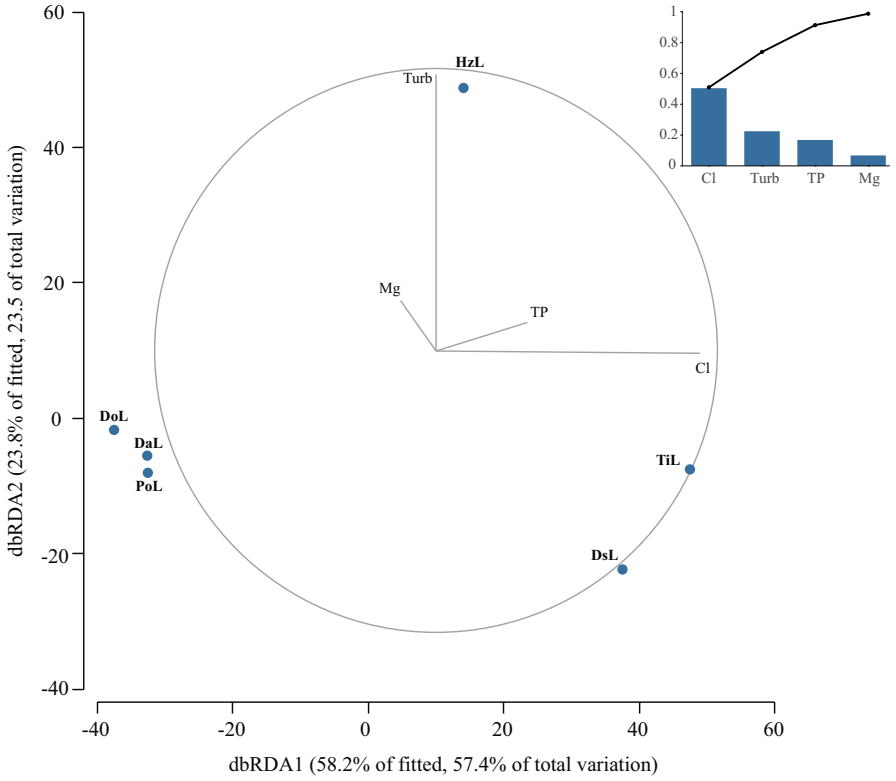

FIGURE 3 dbRDA analysis plot showing the relationship between population distribution and environmental variables. Environmental variables are indicated by the grey line pointing in the direction of increasing values, including chloride (Cl), turbidity (Turb), total phosphorus (TP) and magnesium (Mg). Populations are indicated by blue dots. Blue bar (top-right) represents proportion of explained variation by each environmental variable, and line chart represents cumulative proportion.

in China based on morphological characteristics: *C. fluminea*, *C. nitens*, *C. aurea*, *C. largillierti*, *C. fluminalis*, *C. tenuis* and *C. scholastica* (Wang et al., 2014). *C. fluminea* was described with the broadest distribution, inhabiting most river basins and lakes in China (Shu et al., 2014). Similarly, *C. nitens*, *C. aurea* and *C. largillierti* had a wider distribution than *C. fluminalis* which had only been reported within the Yangtze River Basin (Lin, 1962; Tchang et al., 1965). Specimens of *C. tenuis* and *C. scholastica* had also been detected within the Yuan River and the Huai River respectively (Huang & Li, 2003). High polymorphism and phenotypic plasticity within Corbicula have resulted in the delimitation of distinct morphotypes that appear highly similar genetically, making identification of distinct Corbicula species problematic and often resulting in multiple synonyms for a single lineage (Lin, 1962; Tchang et al., 1965). Similarly, specimens sampled from Dongting Lake, Poyang Lake and Qingshan Lake were previously grouped into two separate subclades and were regarded as *C. fluminea* and *C. leana* (Park & Kim, 2003). However, the macrozoobenthos surveys around these areas only found *C. fluminea* based on morphological examination (Shu et al., 2014).

Although only three major *COI* Corbicula clades were detected by the present study of Chinese lakes and Yangtze River mouth, several distinct *COI* haplotypes were retrieved within clades II and III with 77 distinct *COI* haplotypes in total. Since only the *COI* marker was used here, we did not study the nuclear genetic diversity and we could not detect potential cytonuclear mismatches or hybrids occurring in Corbicula when androgenetic lineages occur in sympatry. The biological species concept, which defines species as units that exhibit barriers to reproduction, is widely used to delimit taxonomical units. The different reproductive strategies found in Corbicula (androgenetic hermaphrodite, sexual dioecism, cross-fertilization and self-fertilization (Gomes et al., 2016; Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014)), as well as possible hybridization between distinct lineages (Hedtke et al., 2008; Lee et al., 2005; Pigneur, Etoundi, et al., 2014; Vastrade et al., 2022), make species delimitation in general using the biological species concept problematic in this genus. Genetic lineages defined using the *COI* marker, as in the present study, can identify distinct mitochondrial lineages within the genus Corbicula as evidenced by other studies (e.g. Park & Kim, 2003; Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014), but species cannot be identified as discussed in Hedtke et al. (2011) or Vastrade et al. (2022) who included nuclear markers and identified discordances due to cross-species hybridizations.

4.2 | Genetic spatial distribution and diversity

The haplotype and nucleotide diversity of sampled Corbicula clams residing within the Yangtze River Basin in the present study ranged between 0.599 and 0.957 and 0.0080 and 0.0464, respectively (Table 3), being higher than in the invasive populations in Europe and America where only four distinct *COI* Corbicula haplotypes were detected: form A/R (FW5), form B (FW1), form Rlc (FW4) and form C/S (FW17) (Gomes et al., 2016; Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014), and recently form D (Haponski & Ó Foighil, 2019). The highest *COI* diversity in our study was detected in Dianshan Lake (DsL) located at the lower reaches of the Yangtze River: 16 distinct haplotypes belonging to clades II (5 haplotypes) and III (11 haplotypes) were found in this lake. Hongze Lake (HzL),

outside but connected with the Yangtze River Basin, also displayed a high diversity with haplotypes of the three clades found in this lake. A similar pattern of *COI* genetic diversity was found in the Corbicula populations of Ebro River (Iberian Peninsula, Europe), with higher haplotype and nucleotide diversity in estuarine populations than inland populations, showing an increased diversity downstream (Peñarrubia et al., 2017). The *COI* haplotype and nucleotide diversity of Dongting Lake (DoL) in the present study were similar to those evaluated using the *COI* marker by Wang, Li, et al. (2018) but higher than those assessed by the *Cytb* marker (Wang, Zhu, et al., 2018), which may be attributed to differences in molecular markers or sampling. The *COI* genetic diversity of HzL was also similar to previously reported data (Li et al., 2015).

The low genetic divergence of Corbicula clams within their invaded range when compared to the native regions likely results from either a founder effect, whereby only a limited number of specimens were introduced from their native range, or through a short-lived bottleneck event followed by a fast population expansion of a few lineages through androgenesis (Pigneur, Etoundi, et al., 2014). The monomorphism found here in clade I (only haplotype 6 = FW5) represents the clonal or androgenetic invasive lineage A/R, which was widespread in the invasive range but also in the native region and within the Yangtze River Basin. Polymorphism in clade II was represented by 36 *COI* haplotypes found in the Yangtze Basin, including the invading lineages B (haplotype 17 or FW1) and Rlc (haplotype 43 or FW4). Invasive lineage B, as abundant as lineage A/R in Chinese lakes, was found in all the lakes sampled in this study except Yangtze River Mouth, while invasive lineage Rlc was sampled in four distinct lakes (Figure 1). A high diversity was also detected in clade III, with 35 distinct *COI* haplotypes retrieved in the Yangtze River and clustering with the brackish sexual species *C. japonica* that was only found in Japan and Korea (Okamoto & Arimoto, 1986; Park et al., 2002). The relatively high *COI* genetic diversity observed in clades II and III could be linked to possible sexual reproduction or frequent origins of asexual Corbicula lineages from sexually reproducing populations (Pigneur, Etoundi, et al., 2014). Biflagellate sperm is a diagnostic marker of androgenesis in Corbicula (Pigneur et al., 2012), and previous reports detected it in Corbicula populations in the middle and upper Yangtze River (Qiu et al., 2001; Wang, Li, et al., 2018), but not in population in the Yangtze River mouth (Zhan, 2020). This spatial variation of reproduction may provide indirect evidence to explain genetic diversity differences.

4.3 | Radiation of Corbicula from marine into freshwater habitats

Previous studies on the phylogeny of the family Cyrenidae revealed invasions of freshwater environments by marine/brackish Corbicula lineages (Glaubrecht et al., 2006; Graf, 2013). Similar results were detected by the present study, supporting the hypothesis of Corbicula radiation from marine into freshwater habitats. First, clade III was mostly found in estuarine lakes such as Tai (TaL) and Dianshan (DsL) lakes in the lower reaches of the Yangtze River Basin (where the chloride concentration was 43.79 ± 15.49 mg/L and 63.68 ± 16.22 mg/L, respectively), as well as the Yangtze River mouth. Both lakes have originated from the closely residing East China Sea over time. As a geologically recent waterbody, Tai Lake was a large embayment of the East China Sea as recent as 1 million years ago and gradually became separated, while Dianshan Lake remains connected with the East China Sea through the Huangpu River (Gu et al., 2019). Two haplotypes of clade III also invaded Chao Lake (ChL) where the chloride is much lower (21.96 ± 4.07 mg/L), while distinct haplotypes of clade III (10 haplotypes in total) invaded Hongze Lake (HzL) where the chloride reaches 42.91 ± 8.83 mg/L. Second, marginal tests resulting from DistLM analysis of *COI* population genetic variation at the regional scale suggested that environmental parameters largely explained the spatial *COI* haplotype distribution, with chloride levels explaining most variations. We, however, did not study the nuclear genetic diversity of Corbicula and therefore the impact of chloride on the spatial genetic variation within the Yangtze River Basin should be considered preliminary. At the global scale, the temperature has been suggested as the critical factor shaping Corbicula distribution (Crespo et al., 2015; Gama et al., 2016). However, at local scales, salinity is considered a major abiotic factor influencing the success and velocity of invasion into new environments in Corbicula as suggested also by other studies (Crespo et al., 2017; Sousa et al., 2006). Notably, salinity has been observed to directly affect the physiological processes of Corbicula (Baba et al., 1999; Crespo et al., 2017). Third, the two invasive lineages A/R and B, abundant in the Chinese lakes studied here, were sampled from freshwater and estuarine lakes (with chloride ranging from 4.58 ± 1.17 to 42.91 ± 8.83 mg/L for lineage A/R and 4.58 ± 1.17 to 63.68 ± 16.22 mg/L for lineage B). Invasive lineage Rlc was found in lakes where chloride ranged between 5.31 mg/L and 42.91 mg/L. The three invasive lineages, therefore, seem to show a wide tolerance towards varying salinity levels, explaining their abundance and widespread distribution within the Yangtze River Basin (Figure 2; Table 2) and also abroad.

Environmental factors other than salinity may have shaped the colonization of clams during dispersal. High turbidity has the potential for decreased feeding, reduced reproduction and even to reduce shellfish survival (Avelar et al., 2014; Tuttle-Raycraft & Ackerman, 2020; Wilber & Clarke, 2001). Besides, as filter-feeding bivalves, the survival of Corbicula depends heavily on the abundance of some phytoplankton species (Sousa et al., 2014). The availability of phosphorus is an important factor that directly controls the phytoplankton growth, biomass and species composition (reviewed in Xu et al., 2010), and therefore may indirectly affect Corbicula populations. Finally, Mg, being essential for the shell formation of Corbicula (Zhao et al., 2017), could also impact the distribution of Corbicula clams. Although these factors may only have a low contribution to the spatial distribution of clams when compared to salinity, their impact should be considered in further analyses.

Environmental factors may vary with regional changes, but salinity usually varies with distance from the estuary, both in invaded and native areas. Salinity was observed here to have the highest impact

on the success of Corbicula radiation and may support genetic barriers that shape an uneven distribution through the limitation of gene flow among different Corbicula lineages, depending on their salinity tolerance (e.g. Crespo et al., 2017). The present study, however, emphasizes that invasive Corbicula lineages found in Europe and America, are also abundant in the native range, combining an androgenetic mode of reproduction with a high salinity tolerance, corroborating their high invasion capacity.

4.4 | Implications for freshwater biodiversity conservation

Control and management of invasive species are highlighted as a priority for biodiversity conservation (Coughlan et al., 2020; Guareschi et al., 2021). Corbicula lineages A/R and B are abundant and widely distributed in native and invaded regions (Gomes et al., 2016; Park & Kim, 2003; Peñarrubia et al., 2017; Pigneur, Etoundi, et al., 2014), and their peculiar reproductive mode (including androgenesis, self- and cross-fertilization) may be one of the main reasons for their invasive capacities in freshwater ecosystems (Gomes et al., 2016; Kraemer et al., 1986; Lee et al., 2005; Vastrade et al., 2022). Importantly, our findings here revealed that these two lineages had a wide tolerance to salinity being able to colonize from estuary to inland rivers and lakes, increasing their invasion potential in different water systems. Conservation managers should pay close attention to populations inhabiting environments with a high or fluctuating salinity, as these populations may cryptically radiate upstream into freshwaters overtime. However, whether those further upstream can disperse in a downstream direction and show tolerance to increased salinity levels still requires further investigation to confirm. Distribution and dispersal modelling for these invasive lineages should be updated to reflect this improved understanding of Corbicula tolerance to salinity, as study findings based on field data corroborate and expand upon what has so far only been observed under laboratory conditions. Therefore, the two invasive lineages require special caution to control and manage, as these lineages have the potential to establish a new population through only one individual (reviewed in Pigneur et al., 2012), cause biomass and production reduction in primary producers but also habitat competition with native species in the invaded ecosystems (Pigneur, Falisse, et al., 2014; Sousa et al., 2014; Strayer, 2010). Given that these two lineages have a high capacity for invasion, it is critical to detect them early during their invasion so that the loss of native species and the cost of management could be reduced. Although detecting Corbicula at an early stage is difficult due to few individuals and small larvae, techniques such as environmental DNA provide a viable method to achieve rapid and sensitive detection (Simberloff et al., 2013). Design of suitable detection primers and kits in the future would directly benefit the biodiversity conservation in invasive areas, as well as the implementation of improved biosecurity protocols (Coughlan et al., 2020).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Newly generated COI haplotype data in this study were deposited in NCBI under the accession numbers ON504951-95, see the Supporting Information Table S3 for details. Additional files could be found in the Supporting Information and accessed on a figshare deposit: https://doi.org/10.6084/m9.figshare.21154609.

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BIOSKETCH

The research team is made up of researchers focusing on the evolutionary biology and ecology of invasive Corbicula complex. We aim to increase understanding of the relationships between environmental factors and Corbicula's adaptation, genetic diversity and reproductive modes.

Author contributions: DLL and CZ conceived the idea. CZ and YXT performed data analysis and draft the manuscript. MV conducted data quality control, verified *COI* data analysis and revised the manuscript. NEC revised and improved the manuscript. KVD verified *COI* data analysis and revised the manuscript. TZ took part in data analysis and manuscript drafting. YJC measured and analysed environmental data. All authors contributed critically to the drafts and gave final approval for publication.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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