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Inferring the invasion mechanisms of the red swamp crayfish in China using mitochondrial DNA sequences

Gen Hua Yue [a,b,c,*], Jian-Bin Feng [d], Jun Hong Xia [e], Su Yin Cao [f], Chun Ming Wang [g]

[a] Molecular Population Genetics and Breeding Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604, Republic of Singapore
[b] Department of Biological Sciences, National University of Singapore, 14 Science Drive, 117543, Republic of Singapore
[c] School of Biological Sciences, Nanyang Technological University, 6 Nanyang Drive, 637551, Republic of Singapore
[d] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201306, China
[e] School of Life Sciences, Sun Yat-sen University, 135 Xingang West, Guangzhou, 510275, China
[f] Animal Science and Technology College, Beijing University of Agriculture, Beijing, 102206, China
[g] College of Agriculture, Nanjing Agricultural University, Nanjing, 210095, China

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ABSTRACT

The red swamp crayfish, *Procambarus clarkii*, is a native species in north-eastern Mexico and south-central USA. *P. clarkii* was introduced to China in 1929 and has been used as an aquaculture species in China since 1983. It currently exists in most of the provinces of China, but threatens local fish, crustaceans, aquatic plants and local freshwater ecosystems. We examined the genetic variation in partial mitochondrial ND2 gene of 831 individuals collected from 25 *P. clarkii* populations in 13 provinces of China to infer the expansion pathways and mechanisms. Six haplotypes were detected. All six haplotypes appeared in four populations in Nanjing and a population located near Nanjing whereas only 1–5 of the six haplotypes were present in other populations. These data suggest that the populations in Nanjing are probably the source of all other populations in China. There were no significant relationships between geographic distances and genetic distances in 25 populations, whereas significant relationship was found in four populations in Qinhuai River covering 50 km in Nanjing. These data suggest that the expansion mainly be human-mediated in large scale, and active disposal or non-anthropogenic passive dispersal might have played an important role in expansion at a smaller scale. In some places far away from Nanjing, several haplotypes existed, suggested multiple introduction events may have happened. Although aquaculture of this species could bring huge economic benefit, its potential to negatively affect native biota and entire ecosystems should not be ignored.

1. Introduction

Alien invasive species are one of the major contemporary threats to global biodiversity. On a global scale, the introduction of exotic species can have serious and long-lasting effects on ecological interactions, biodiversity and fisheries and may facilitate invasion of other non-native species (Ruiz & Carlto, 2003; Williamsom, 1998). Non-native species cost approximately US$120 billion/year in damage and control (Pimentel, Zuniga, & Morrison, 2005).

A crucial factor in the control and management of the spread of invasive species is to identify the source populations and to determine the mechanisms of spread. Identifying the sources of an invasive population may be useful in determining the most important transport vectors responsible for bringing the organisms to new areas (Ruiz & Carlto, 2003). Understanding the biology and ecology of an invasive species in its native habitat can provide clues on controlling the damage and predicting potential invasion ability in a new area (Ficetola, Bonin, & Miaud, 2008). Determining the invasion pathways can lead to the deeper understanding of the mechanisms underlying a successful invasion (Corin, Ritchie, & Lester, 2008; Durka, Bossdorf, Prati, & Auge, 2005). In practice, however, it is difficult to know the routes of spread of an invasive species as field observations are usually unable to identify source populations, multiple introductions, and cryptogeneic taxa and can quantify the genetic variation available in the invading population

* Corresponding author. Molecular Population Genetics and Breeding Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604, Republic of Singapore.
E-mail address: genhua@tll.org.sg (G.H. Yue).

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(Ruiz & Carlto, 2003). In recent years, due to the rapid development of molecular biology and sequencing technologies, many molecular genetic tools, such as microsatellites, mitochondrial DNA sequences and SNPs have been developed to provide means to follow the invasion pathways of invasive alien species (Chen et al., 2017; Darling & Blum, 2007; Okada, Ahmad, & Jasieniuk, 2007). Molecular tools (Shen & Yue, 2019) can be used to determine the genetic variation and population structure in native habitats and invasion areas (Cameron, Bayne, & Coltman, 2008; Gu, Wang, Li, Li, & Shen, 2020). This information further can be used to infer the source population of introduced populations (Freshwater et al., 2009; Le Roux & Rubinoff, 2009; Zidana, Turner, Van Oosterhout, & Hanfling, 2009) and to deduce the pathways of the invasion (Cameron et al., 2008; Cheng, Cheng, Xu, & Xie, 2008; Corin et al., 2008; Provan, Booth, Todd, Beatty, & Maggs, 2008).

The red swamp crayfish (*Procambarus clarkii*, Girard, 1852) is native to south-central United States and north-eastern Mexico (Huner, 1988) and has been found in ponds, ditches, marshes, rivers, slow flowing water, reservoirs, irrigation systems and rice fields. *P. clarkii* has been introduced to Europe, Africa, central and south America and southeast Asia (Cruz & Rebelo, 2007; Gherardi, 2006; Loureiro, Anastácio, Araujo, Souty-Grosset, & Almerão, 2015; Mkoji et al., 1999; Putra et al., 2018). When introduced into a suitable habitat, *P. clarkii* can become established and eventually becomes a dominant species. Its introduction may cause dramatic changes in native plant and animal communities (Li, Dong, Li, & Wang, 2007; Li & Xie, 2002; Rodriguez, Becares, Fernandez-Alaez, & Fernandez-Alaez, 2005). *P. clarkii* also reduces the value of the freshwater habitats in which it occurs by consuming invertebrates and macrophytes and degrading river banks by its burrowing activity (Holdich, Gydemo, & Rogers, 1999). Some crayfish plague, including the oomycete *Aphanomyces*, was carried and transmitted by *P. clarkia* and caused diseases in local crayfish species (Aquiloni, Martin, Gherardi, & Diéguez-Uribeondo, 2011; Diéguez-uribeondo & Söderhäll, 1993). According to the historical record, *P. clarkii* was introduced once to Nanjing, Jiangsu province, China from Japan in 1929 (Li et al., 2007; Li & Xie, 2002). Since 1983, *P. clarkii* has been cultured for food in Nanjing and nearby regions. Translocation of *P. clarkii* from Nanjing and nearby regions to other provinces took place frequently in the past 30 years (Cao, Zhou, & Zhanf, 2010; Wang et al., 2009; Yue, Zhu, Wang, & Feng, 2010). This crayfish can be found in most provinces of China nowadays. Recently, due to huge economic benefit of culturing this crayfish for food (Jin et al., 2019) and for the ornamental fish market (Patoka, Kalous, & Kopecký, 2015), this species has been extensively cultured in many places in China (Jin et al., 2019). However, it has to be noted that *P. clarkii* threatens local fish, crustaceans, aquatic plants and local freshwater ecosystems (Cao et al., 2010; Yue et al., 2010). Several different mechanisms may have played a role in expanding *P. clarkii*'s distribution such as active natural dispersal, escaping from human-mediated translocation and deliberate introduction by human for food and ornamental fish market. Yet the mechanism playing the major role is not clear. A previous study on six populations of *P. clarkii* in Jiangsu and Zhejiang provinces of China showed that the population in Nanjing displayed the highest genetic diversity, leading to hypothesize that all the populations appearing in other places of China may be originated from the introduced population in Nanjing (Yue et al., 2010). However, this hypothesis has not been fully proved, although currently, there is more evidence supporting this hypothesis (Li et al., 2012; Li et al., 2015).

In this study, we used one of the most variable gene in the mitochondrial genome: the partial mitochondrial NADH dehydrogenase subunit 2 (ND2) to assess the levels and patterns of genetic diversity present in 25 populations of *P. clarkii* collected in 13 provinces of China. Two scenarios were tested: 1) the population in Nanjing may be the source of all other populations in China and 2) the expansion may mainly be human-mediated in large scale, whereas active dispersal or non-anthropogenic passive dispersal might have played an important role in expansion at a smaller scale. The purpose of this study was to know the potential invasion routes of *P. clarkii* and the forces leading to its expansion in China. We believe that although the aquaculture of this crayfish for food and ornamental crayfish could bring huge economic benefit, measures must be taken to prevent its threats to local freshwater ecosystems and local crayfish species because there is also indigenous crayfish *Cambaroides dauricus* in north-eastern China. This crayfish is sensitive to crayfish plague pathogens, which are transmitted by *P. clarkia* (Wang & Cui, 2007).

2. Materials and methods

2.1. Sampling and DNA extraction

A small piece of the third pleopod from each of 831 individuals of *P. clarkii* was collected from 25 populations located in 13 provinces of China (see details in Table 1 and Fig. 1). In Nanjing (the potential source population of other populations in China), 149 samples were collected from four locations (NJ1, NJ2, NJ3 and NJ4) in Qinhuai River covering about 50-km to study the small-scale dispersal. Similarly; to study the dispersal in microenvironment in other locations, four populations (TX, HZ, HN and WJ) covering 150 km in Zhejiang Province (Fig. 1, B) and four populations (ZJ, WX, SZ and SJ) covering 300 km in Jiangsu Province (Fig. 1 C) were collected. DNA was extracted from tissues using a method developed by us (Yue & Orban, 2005).

2.2. PCR amplification and sequencing of part of the mitochondrial ND2 gene

Partial sequence of *P. clarkii* mitochondrial ND2 (AF436024) was downloaded from GenBank. Primers (Pcl-ND2-F: GAAGGTTTACCTCCTTTTTTAGG; Pcl-ND2-R: GTGGAGAAAAGTCATCGTTTCGT)

Table 1
Information on 831 samples collected in 25 *Procambarus clarkii* populations including the collection location, the sample abbreviation and the time period of sampling.

Sample location	Sample abbreviation	Date sampled	Samples sequenced
Nanjing-1, Jiangsu Province	NJ1	2003	35
Nanjing-2, Jiangsu Province	NJ2	2003	40
Nanjing-3, Jiangsu Province	NJ3	2003	36
Nanjing-4, Jiangsu Province	NJ4	2003	38
Suzhou, Jiangsu Province	SZ	2004	33
Wuxie, Jiangsu Province	WX	2004	27
Zhengjiang, Jiangsu Province	ZJ	2004	38
Jiangdu, Jiangsu Province	JD	2004	32
Xieyi, Jiangsu Province	XY	2004	38
Songjiang, Shanghai/ Jiangsu Province	SJ	2004	34
Chuxian, Anhui Province (North side)	AH1	2005	22
Xianchen, Anhui Province (South side)	AH2	2005	28
Huzhou, Zhejiang Province	HZ	2004	35
Tongxiang, Zhejiang Province	TX	2003	39
Haining, Zhejiang Province	HN	2009	40
Wujiang, Zhejiang Province	WJ	2009	35
Nanchang, Jiangxi Province	NC	2008	34
Guangzhou, Guangdong Province	GZ	2008	35
Fuzhou, Fujian Province	FJ	2009	35
Changde, Hunan Province	HUN	2008	32
Wuhan, Hubei Province	WH	2006	31
Xinyang, Henan Province	HEN	2008	32
Shijiazhuang, Hebei Province	HEB	2008	32
Jining, Shandong Province	SD	2009	20
Zhigong, Sichua Province	SC	2006	30

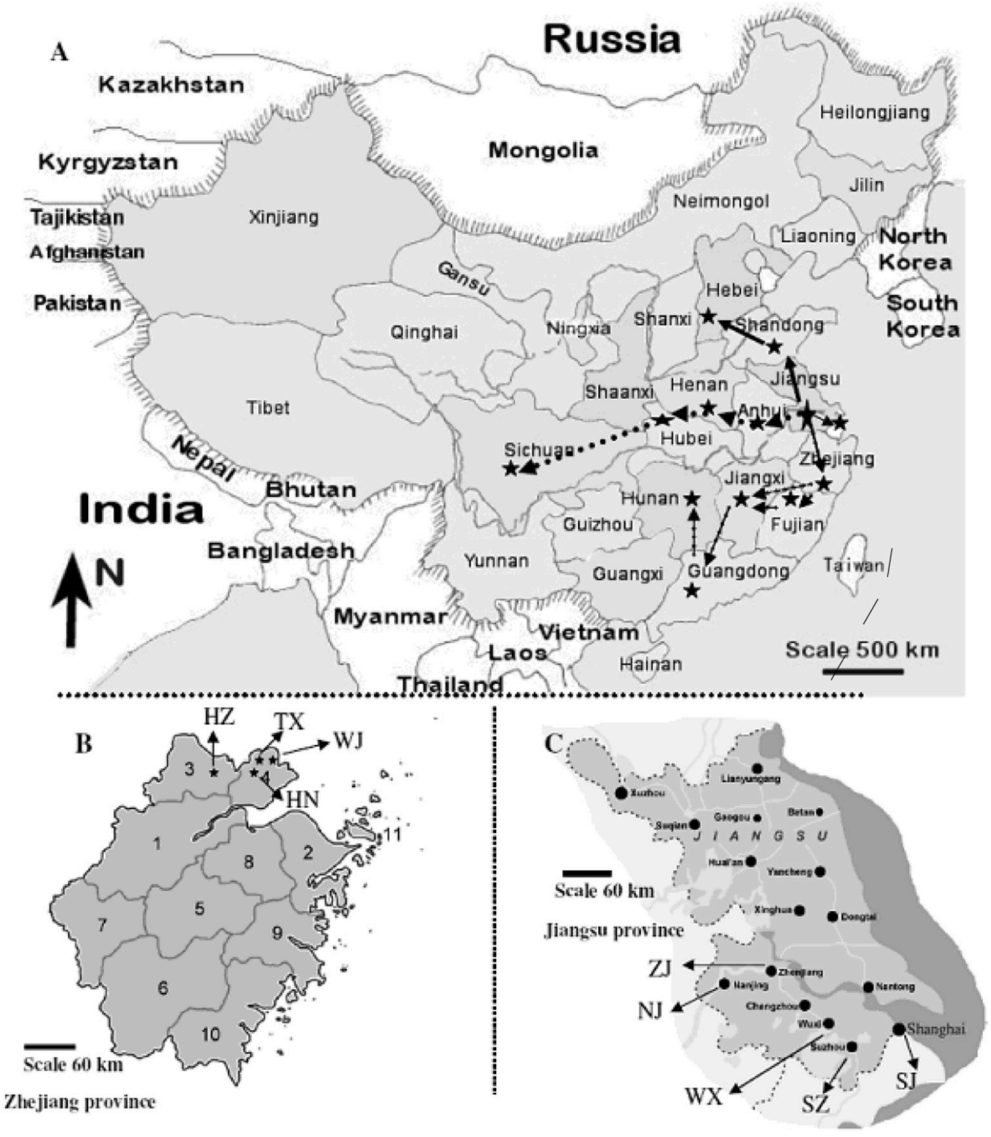

Fig. 1. Locations of sampling of *P. clarkii* in China. A: Map of China showing the 13 provinces (each labelled with a star) where *P. clarkii* samples were collected and three potential major pathways (labelled different types of arrows) of expansion of *P. clarkii* in China; B. Four sample locations (HZ, TX, WJ and HN) in Zhejiang Province and C. Five sampling locations ZJ, NJ, WX, SZ and SJ in Jiangsu Province.

were designed to amplify a fragment of 492 bp using PrimerSelect (DnaStar, MA, USA) software. PCR was conducted on a PTC-100 PCR machine (MJ research) using the following PCR program: initial denaturation at 94 °C for 2 min followed by 36 cycles of denaturation at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s and a final extension at 72 °C for 10 min. PCR was conducted in a total volume of 25 μL, containing 40 ng DNA, 1 × PCR buffer (Finnzymes, Espoo, Finland) with 1.5 mmol/L $MgCl_2$, 50 nmol/Lof each primer, 50 μmol/L of each dNTP and one unit of DNA polymerase (Finnzymes, Espoo, Finland). PCR products were examined on 2% agarose gels and cleaned using GFX columns (Amersham Biosciences, Little Chalfont, United Kingdom). PCR products of each individual were sequenced in both 5′ and 3’ directions using BigDye chemicals, Pcl-ND2-F and Pcl-ND2-R primers on an ABI3730xl DNA sequencer (Applied Biosystems, CA, USA). Forward and reverse sequences were assembled using Sequencher v4.9 (GeneCodes, MA, USA) software.

2.3. Data analysis

Sequences of all individuals were aligned using CLUSTALX (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) software. The following parameters were calculated: the number of haplotypes (n), the nucleotide diversity (π) and the gene diversity (H) using software DNASP v5 (Librado & Rozas, 2009). To illustrate the relationship among different haplotypes, a haplotype network was constructed using the statistical parsimony method in the program TCS (Clement, Posada, & Crandall, 2000). An analysis of molecular variance (AMOVA) was performed using ARLEQUIN (Excoffier, Laval, & Schneider, 2005) to test the distribution of molecular variance among populations. Pairwise genetic distances among populations were also calculated with ARLEQUIN using F_{ST}. The isolation-by-distance model offers an empirical means to test pattern of measures of population subdivision. Sub-divided natural populations that fit one dimensional diffusive stepping-stone model should exhibit a strong fit to the isolation-by-distance model (Russell et al., 1997). Correlation between genetic and geographic distances was assessed using IBDWS v3.08 (Jensen, Bohonak, & Kelley, 2005). Significance of the analysis was examined using Mentel tests over population pairs as implemented in software IBDWS. Finally, the four populations (NJ1, NJ2, NJ3 and NJ4) located in Nanjing were examined for genetic evidence of population growth by performing mismatch

distribution analysis (Rogers & Harpending, 1992) and calculating Harpending's raggedness index (Harpending, 1994) using ARLEQUIN (Excoffier et al., 2005).

3. Results

A fragment of the mitochondrial ND2 gene spanning 492 bp was PCR amplified and sequenced for 831 individuals from 25 locations in China. Analysis of DNA sequences yielded six haplotypes (GenBank accession nos: GU980852-GU980857, Table 2). The haplotype H4 was most frequent (624/831), appearing in all 25 populations, followed by H1 (145/831) present in 19 populations, whereas frequencies of haplotypes H2, H3, H5 and H6 were relatively low appearing only in NJ1, NJ2, NJ3, NJ4, ZJ and some other populations (e.g. XY, JD, TX and AH1).

All six haplotypes were present in the four populations (NJ1, NJ2, NJ3 and NJ4) located in Nanjing and a population (ZJ) near Nanjing, while in other populations, only one to five haplotypes were found (Table 2). The overall haplotype diversity and nucleotide diversity were 0.423 and 0.0011 respectively, while the values within population ranged from zero to 0.612 and 0.0008–0.0025 respectively. The four populations (NJ1, NJ2, NJ3 and NJ4) showed the highest number of haplotypes ($n = 6$), haplotype diversity (H = 0.576–0.619) and nucleotide diversity ($\pi = 0.0023$–0.0025) followed by the populations ZJ ($n = 6$, H = 0.528 and $\pi = 0.0021$), AH1 (n = 5, H = 0.597 and $\pi = 0.0030$) and XY ($n = 5$, H = 0.512 and $\pi = 0.0015$). In the six populations located far away from Nanjing (SC, HUN, WH, HEN, HEB and SD), only the haplotype H4 appeared, whereas in other populations (i.e. HZ, TX, HN, WJ, SZ, WX, SJ, NC, GZ, FJ, JD, XY, AH1 and AH2), only 2–4 haplotypes were present.

Examination of the evolutionary relationships among the six haplotypes revealed a pattern radiating from a central haplotype H4 (Fig. 2). The analysis of mismatch distribution showed that in all four populations located in Nanjing, simulated mismatch values were not significantly different from the observed values under sudden expansion model. In addition, Harpending's raggedness index was consistent with the sudden expansion model for all four populations (Table 3). All these tests indicate population expansion of the four populations located in Nanjing.

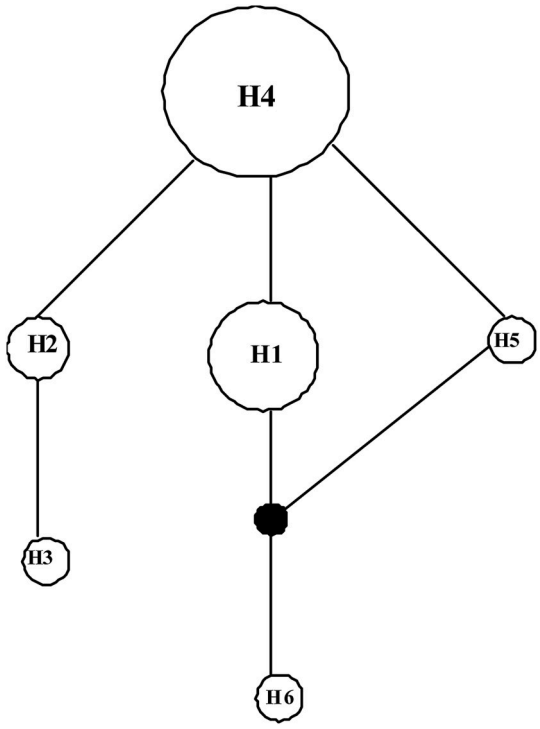

Fig. 2. Mitochondrial haplotype network in *P. clarkii* in China. The areas of circles are proportional to the number of samples of each haplotype. The lines represent single nucleotide mutations and black circle represents the haplotype not seen in current study.

The AMOVA showed that the genetic variance among populations explained 8.27% of the total variance, while the genetic variance within populations accounted for 91.73% of total genetic variance. Pair wise F_{st} analysis showed that genetic differentiation was significant ($P < 0.05$)

Table 2
Haplotypes, their frequency and diversity in 25 *P. clarkii* populations.

Population	N	N						H ± Se	π
		H1	H2	H3	H4	H5	H6		
NJ1	35	6	1	1	22	1	4	0.576 ± 0.085	0.0023
NJ2	40	9	2	3	23	2	1	0.619 ± 0.069	0.0024
NJ3	36	6	2	1	22	2	3	0.602 ± 0.084	0.0025
NJ4	38	6	2	2	24	1	3	0.579 ± 0.085	0.0024
SZ	33	4	0	0	25	2	2	0.417 ± 0.100	0.0013
WX	27	6	0	0	21	0	0	0.389 ± 0.091	0.0007
ZJ	38	5	2	2	24	4	1	0.528 ± 0.085	0.0021
JD	32	5	2	0	25	0	0	0.373 ± 0.096	0.0008
XY	38	6	2	2	26	2	0	0.512 ± 0.089	0.0015
SJ	34	9	0	0	25	0	0	0.401 ± 0.073	0.0008
AH1	22	6	1	1	13	1	0	0.597 ± 0.091	0.0030
AH2	28	9	0	0	19	0	0	0.476 ± 0.057	0.0010
HZ	35	9	0	0	26	0	0	0.393 ± 0.073	0.0008
TX	39	8	3	0	28	0	0	0.448 ± 0.081	0.0010
HN	40	12	0	0	28	0	0	0.431 ± 0.060	0.0009
WJ	35	9	4	0	22	0	0	0.504 ± 0.071	0.0012
NC	34	15	0	0	19	0	0	0.508 ± 0.029	0.0010
GZ	35	12	0	0	23	0	0	0.464 ± 0.054	0.0005
FJ	35	3	0	0	32	0	0	0.161 ± 0.079	0.0003
HUN	32	0	0	0	32	0	0	0	0
WH	31	0	0	0	31	0	0	0	0
HEN	32	0	0	0	32	0	0	0	0
HEB	32	0	0	0	32	0	0	0	0
SD	20	0	0	0	20	0	0	0	0
SC	30	0	0	0	30	0	0	0	0
Overall	831	145	21	12	624	15	14	0.423 ± 0.020	0.0011

N: number of samples, n: number of haplotypes, H: haplotype diversity and π: nucleotide diversity.

Table 3
Result of population expansion tests on the four populations of *Procambarus clarkii* in Nanjing.

Population	Sudden expansion model *P* value	Harpending's raggedness index	*P* value
NJ1	0.10	0.079	1.00
NJ2	0.24	0.102	0.19
NJ3	0.54	0.074	0.80
NJ4	0.60	0.061	0.78

among most populations, while the genetic differentiation among four populations (NJ1, NJ2, NJ3 and NJ4) collected in Qinhuai river located in Nanjing was not significant ($P > 0.05$) (see details in Supplementary material 1).

The Mentel tests demonstrated that there were significant relationships between genetic distances and geographical distances ($r = 0.86$, $P < 0.01$) among four populations (NJ1, NJ2, NJ3 and NJ4) located in Qinhuai River covering 50 km in Nanjing, while the relationships between genetic distances and geographical distances among four populations ($r = 0.36$, $P > 0.05$) in Jiangsu province and four populations ($r = -0.03$, $P > 0.05$) in Zhejiang province were statistically not significant. In all 25 populations, there were no significant relationships between genetic and geographical distances ($r = 0.33$, $P > 0.05$).

4. Discussion

According to our best knowledge, this study is the first detailed genetic analysis of *P. clarkii*, covering 13 provinces of China although some studies on the population genetics of this species in a few provinces have been conducted (Barbaresi, Fani, Gherardi, Mengoni, & Souty-Grosset, 2003; Cao et al., 2010; Wang et al., 2009; Yue et al., 2010); (Li et al., 2012; Li et al., 2015). In all 831 individuals, only six haplotypes were detected; the number of haplotypes, haplotype diversity and nucleotide diversity in all 25 populations of *P. clarkii* were lower than in central and south America (Torres & Álvarez, 2012) and in introduced populations of *P. clarkii* in Europe (Barbaresi, Gherardi, Mengoni, & Souty-Grosset, 2007). This data is in agreement with results of our previous study showing low genetic diversity in six populations analysed using microsatellites (Yue et al., 2010). This low level of sequence divergence is expected, as the introduction of *P. clarkii* from Japan was only once and colonization of *P. clarkii* is quite recent (<85 years) (Li et al., 2007). Similar low genetic variation in alien invasive species as compared with those in source populations have been described in a number of species, such as the introduced red swamp crayfish (<40 years) in Europe (Barbaresi et al., 2007; Barbaresi et al., 2003) and Egypt (Radwan, Hassan, El-Aziem, & Abbass, 2014) and the introduced Chinese mitten crab (>100 years) in Europe (Herborg, Weetman, Van Oosterhout, & Hanfling, 2007).

The sampling area of this study is much larger than in previous studies on genetic diversity of *P. clarkii* (Barbaresi et al., 2007; Cao et al., 2010; Li et al., 2012; Li et al., 2015; Wang et al., 2009; Yue et al., 2010); therefore the low genetic variation found in this study may reflect the actual genetic status of *P. clarkii* in China. All six haplotypes appeared in four populations (NJ1, NJ2, NJ3 and NJ4) in Nanjing and a population (ZJ) near Nanjing (about 70 km away), while in all other populations only one to five haplotypes were detected. In six populations (SC, HUN, WH, HEN, HEB and SD), only the most frequent haplotype H4 was detected. All haplotypes that appeared in other populations were found in the four populations in Nanjing. The mitochondrial haplotype network showed a star-like pattern, suggesting that the four populations located in Nanjing have undergone a population expansion (Avise, 2000). Furthermore, the analysis of mismatch distribution and Harpending's raggedness index indicated population expansion of *P. clarkii*. The mitochondrial haplotype network showed that the haplotype H4 was the most frequent, suggesting H4 is the most ancestral haplotype. Although high genetic variation of invasive species could be caused by multiple introductions from source populations (Andreakis, Kooistra, & Procaccini, 2009; Tang et al., 2009; Zidana et al., 2009), the higher genetic diversity present in four populations in Nanjing might not be caused by multiple introductions, as according to the historical record, *P. clarkii* was introduced to Nanjing from Japan once in 1929 and translocation of *P. clarkii* from other places to Nanjing for aquaculture has not taken place (Li et al., 2007). Altogether, our data suggest that *P. clarkii* in Nanjing is probably the first introduced population in China (Yue et al., 2010) and support the hypothesis that the populations in Nanjing could have acted as the source of *P. clarkii* for spread to other sites in China.

The AMOVA and F_{ST} analysis showed significant genetic differentiation among most populations, while in the four populations in Nanjing, the genetic differentiation was not significant, suggesting that the four populations in Nanjing could be regarded as one population and a number of discrete introduction events have occurred in large scale. Occurrence of population bottleneck, human-mediated translocation and/or genetic drift of small populations may have contributed to population differentiation in large scale. Analysis of isolation-by-distance in all 25 populations, in four populations in Nanjing, four populations in Jiangsu province and four populations in Zhejiang province revealed that for the four populations collected in the Qinhuai river in Nanjing there were significant relationships between geographical and genetic distances between populations, indicating *P. clarkii* was spreading at smaller scale via active or non-anthropogenic passive dispersal (e.g. by fish and aquatic animals). While in all 25 populations, the four populations in Jiangsu province and four populations in Zhejiang Province, there were no significant relationships between geographical and genetic distances between populations, suggesting besides active dispersal or via non-anthropogenic passive dispersal, other factors must also be taken into account. Since in the past 25 years, culture of *P. clarkii* for food have been intensive in Jiangsu, Anhui and Zhejiang provinces; translocation of *P. clarkii* from Nanjing and neighbouring locations to other locations for aquaculture happened very frequently (Wang et al., 2009; Yue et al., 2010), and cultured *P. clarkii* often escaped to the wild. Furthermore, although it was reported that *P. clarkii* could move 1–11 m/day (Gherardi, Tricarico, & Ilheu, 2002), it is unlikely for *P. clarkii* to move itself over several hundred km within less than 85 years, as continuous movement/migration is impossible and some natural barriers (e.g. land and mountains) prohibit movement. Therefore, human-mediated disposal may have played an important role in the expansion of *P. clarkia* although it is also possible that water birds, including ducks, can transport juvenile red swamp crayfish to distant places (Águas, Banha, Marques, & Anastácio, 2014). In China, several tools have been used to translocate *P. clarkii* for aquaculture and/or for food, such as vehicles on land and boats in rivers. It is not known which vector mainly caused the introduction of *P. clarkii* into new places. We hypothesize that vehicles could be the major vector for the expansion of *P. clarkii* in China as development of road transport was much quicker than that of transport on rivers in the past 20 years. It was reported that dispersal of two freshwater invasive macroinvertebrates, *P. clarkii* and *Physella acuta* could be made by off-road vehicles in Portugal (Banha, Marques, & Anastácio, 2014). However, this hypothesis must be examined by collecting samples near major roads and rivers in order to examine genetic diversity in populations in these locations to infer the major vector (Cameron et al., 2008).

In some populations (ZJ, XY, AH1, JD, TX, WJ and SZ) located in Jiangsu, Zhejiang and Anhui provinces, which are the neighbouring locations of Nanjing, the number of haplotypes, haplotype diversity and nucleotide diversity were only slightly lower than that in the source population in Nanjing. The high genetic variation present in these populations may be caused by single introduction of large number of individuals from the source population in Nanjing or multiple introductions from the source population. In Jiangsu, Zhejiang and Anhui provinces, aquaculture of *P. clarkii* for food is a common practice (Wang

et al., 2009; Yue et al., 2010). Translocations of *P. clarkii* from Nanjing to Jiangsu, Zhejiang and Anhui provinces for aquaculture and food took place frequently. On the other hand, translocations of *P. clarkii* from these provinces to other places for aquaculture and food also took place frequently (Li et al., 2007; Li & Xie, 2002). Therefore, these populations could have severed as secondary source populations for expanding to other places. In some populations (SC, HUN, WH, HEN, HEB and SD) located far away from the first introduced population in Nanjing, only one haplotype H4 existed, suggesting sever bottlenecks in these populations. This result is not surprising, as a number of previous studies showed that invasive populations contained lower genetic variation as compared to source populations (Drescher, Bluthgen, & Feldhaar, 2007; Kauserud et al., 2007; Peacock, Beard, O'Neill, Kirchoff, & Peters, 2009). Genetic theory predicts that when a new population is founded by a subset of individuals from a source population, the genetic diversity in the introduced populations is usually lower than in the source population (Nei, Maruyama, & Chakraborty, 1975). It is most likely that the populations of SC, HUN, WH, HEN, HEB and SD expanded from one single introduction by human-mediated translocation from nearby populations. However, these populations could also have arisen as results of multiple introductions of the same haplotype H4 from various sources, as the haplotype H4 was present in all 25 populations studied. Although aquaculture of *P. clarkii* in China had bought huge economic benefit, people have to note that this species is not a native species to the freshwater ecosystems of China and it could threat the local fish, crustaceans and aquatic plants. Therefore, it is essential to take measures to prevent to its escape from culturing places to local freshwater systems. It should not be ignored that the trade of the *P. clarkii* as pet in the ornamental fish market could be also a cause of increase risks of harmful invasions of alien species (Patoka et al., 2018). Therefore, prevention of the release of pet *P. clarkii* to freshwater systems is also essential.

In conclusion, our genetic data covering large areas in China suggest that the *P. clarkii* population in Nanjing may be the source of all other populations in China and the expansion may be mainly human-mediated in large scale, whereas active disposal or non-anthropogenic passive dispersal might have played an important role for its expansion at a smaller scale. This novel information about the source, mechanisms of spread and expansion routes of *P. clarkii* will provide an important basis for drawing effective prevention and management strategies. Although the aquaculture of *P. clarkii* for food and ornamental fish market in China could bring huge economic benefit, it is essential to take measures to prevent its threats to local freshwater ecosystems and native fish, crustaceans and aquatic plants.

CRediT authorship contribution statement

Gen Hua Yue: Project administration, Data curation, Formal analysis, Writing - original draft. **Jian-Bin Feng:** Data curation. **Jun Hong Xia:** Data curation. **Su Yin Cao:** Data curation. **Chun Ming Wang:** Data curation.

Declaration of competing interest

The authors declare that there is no Conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aaf.2020.04.003.

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