BIODIVERSITY RESEARCH

Phylogenetic evidence for multiple and secondary introductions of invasive snails: *Pomacea* species in the People's Republic of China

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ABSTRACT

Aim To determine the genetic diversity of invasive snails (*Pomacea* spp.) that are implicated in crop damage, environmental degradation and human disease, and to determine their distribution pattern in a large part of eastern Asia.

Location People's Republic of China (P.R. China).

Methods We collected *Pomacea* snails in a national survey using a grid sampling approach. Overall, 544 snails from 54 sites were used for the present investigation. The mitochondrial *cox1* gene was amplified and sequenced from all the snails. We determined and classified the haplotypes using network analyses and mapped them within P.R. China. Haplotypes from this study, together with sequences available from GenBank, were used to reveal the global distribution of *Pomacea canaliculata* and *P. insularum*.

Results We obtained 521 *cox1* sequences and identified 24 unique haplotypes. Six haplotypes were commonly found in P.R. China. Two species, *P. canaliculata* and *P. insularum*, and one cryptic group were observed. The distribution of the 24 haplotypes across P.R. China shows a mosaic pattern. Globally, only six of 112 haplotypes of *P. canaliculata*, *P. insularum*, *P. dolioides*, *P. lineata* and *P. paludosa* are shared between introduced and native snail populations. We found 16 haplotype clusters, five of which occur in mainland P.R. China. Three of the five clusters could be traced back to South America. The remaining two clusters were unique to P.R. China.

Main conclusions Phylogenetic analyses indicate that *P. canaliculata*, *P. insularum* and two cryptic groups, discovered by the present and previous studies, coexist in the mainland of P.R. China. The mosaic distribution and the high diversity found in the collection sites suggests multiple and secondary introductions. The findings indicate the importance of preventing further intentional introductions and call for appraisal of the risk posed by these snails in vulnerable areas. Discrimination of the ecological impacts of the different species or genotypes will help to develop setting-specific management strategies.

Keywords

Biological invasions, invasive species, mosaic distribution, multiple introductions, People's Republic of China, phylogenetic analysis, *Pomacea* spp.

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Diversity and Distributions

INTRODUCTION

Pomacea is the largest of nine extant genera in the family Ampullariidae (apple snails). There is considerable intraspecific variation in morphology, and hence, it is difficult to determine the true number of species (Cazzaniga, 2002; Hayes *et al.*, 2009). It has been estimated that around 50 species occur in the native range, South and Central America (Cazzaniga, 2002; Cowie *et al.*, 2006). One of them, *Pomacea canaliculata*, has been classified as one of the 100 worst invasive species in the world (Lowe *et al.*, 2000). In around 1980, this snail species was introduced into Southeast Asia, first in Taiwan and subsequently in neighbouring countries (Joshi & Sebastian, 2006). In Asia, 'Golden apple snail' is the most widely used common name for *Pomacea* snails from South America because of their often yellowish shell and their commercial value (Cowie *et al.*, 2006). The initial introduction of this snail into Southeast Asia was intentional, as both a food commodity for export and an important local source of protein. However, several years after the introduction, damage to crops and ecosystems became apparent (Joshi & Sebastian, 2006). Hence, different control strategies have been implemented, but elimination of *Pomacea* snails proved difficult. Among the issues hindering control was the lack of detailed knowledge of species-specific distribution patterns. This challenge has been addressed by a growing number of studies of the morphological and molecular characteristics of *Pomacea*. Results from these studies suggest that four species, namely *P. canaliculata*, *P. insularum*, *P. scalaris* and *P. diffusa*, have been introduced from South America to Asia (Hayes *et al.*, 2008).

In the mainland of the People's Republic of China (P.R. China), *Pomacea* snails were introduced to Zhongshan, Guangdong province, in 1981. Secondary introduction for commercial aquaculture occurred between the mid-1980s and the early 1990s. At present, *Pomacea* snails are established in at least 11 provinces (Lv *et al.*, 2009b), and their range is predicted to expand northward because of environmental transformation and climate change (Lv *et al.*, 2011). Costs and benefits for humans and the environment because of these invaders are difficult to estimate. Additionally, *Pomacea* snails have been identified as a major intermediate host of *Angiostrongylus cantonensis*, the rat lungworm, which infects humans and has driven the emergence of human eosinophilic meningitis in P.R. China (Lv *et al.*, 2008, 2009b). *Pomacea* snails are amphibious and show a preference for shallow ditches and ponds surrounding human settlements, which facilitate contact with peridomestic rats (e.g. *Rattus norvegicus* and *R. rattus*), the most common definitive hosts of *A. cantonensis*. Rats are important predators of *Pomacea* snails (Yusa *et al.*, 2006). Native snail species such as *Cipangopaludina* spp. and *Bellamya* spp., which are commonly consumed in P.R. China, are rarely infected with *A. cantonensis*, although infections and considerable worm loads have been observed under experimental conditions (Lv *et al.*, 2006). Ecological characteristics (e.g. their benthic

habit) probably account for the low likelihood of *A. cantonensis* infection in these native snail species.

Apple snails have now been spreading across P.R. China for three decades. However, the first national survey of their distribution was not performed until 2006 (Lv *et al.*, 2009b). Knowledge gaps still remain with regard to the number and identity of the species and their patterns of spread. Various scientific names, including *Ampullaria crosseana*, *Ampullaria gigas*, *Pomacea canaliculata*, *Pomacea lineata* and *Pila gigas*, have been used to refer to them and have caused considerable confusion (Cowie *et al.*, 2006; Zhou *et al.*, 2009). The external morphology (e.g. shell-banding pattern and foot colour) was initially considered as characteristic for different species, but its usefulness in distinguishing species proved unreliable (Matsukura *et al.*, 2008). Recently, molecular methods [e.g. mitochondrial DNA sequence and inter-simple sequence repeat (ISSR)] have been employed to reveal the diversity of *Pomacea* spp. (Xu *et al.*, 2008; Song *et al.*, 2010; Dong *et al.*, 2011). These studies confirmed the presence of two species, *P. canaliculata* and *P. insularum*. However, their exact distribution patterns remain to be elucidated.

In the present study, we sequenced the mitochondrial gene *cox1* from snail samples collected across the range of *Pomacea* in P.R. China to (1) confirm the number of *Pomacea* species in the mainland of P.R. China, (2) assess the genetic diversity and (3) reveal the distribution pattern of these invasive species. The implications of the results for management and conservation are discussed.

METHODS

Snail collection and total DNA extraction

Pomacea spp. were collected during the first national survey pertaining to *A. cantonensis* in the mainland of P.R. China, in 2006 and 2007 (Lv *et al.*, 2009b). The area where *A. cantonensis* was thought to occur was overlaid with a grid of 164 40 km × 40 km squares. Snail searches were undertaken in each grid square with *Pomacea* spp. found in 54 of them. Approximately 100 *Pomacea* snails were collected from each of the 54 sites. Whenever possible, 10 specimens were selected per site and preserved in 70% ethanol after examination for *A. cantonensis* larvae. In total, 544 specimens from the 54 sites were used for the present study.

From each specimen, *c.* 10 mg of foot tissue was washed several times using PBS buffer and then immersed in the same buffer overnight to expel any remaining ethanol. The foot tissue was cut into small pieces and incubated with sodium dodecyl-sulphate/proteinase K at 56°C for 4–6 h. The suspension was centrifuged for 60 s at 10,000 × *g* and the supernatant transferred to a clean tube and DNA extracted with phenol/chloroform/isoamyl alcohol (v:v:v = 25:24:1). The DNA pellet was suspended in 30–50 μl H_2O and kept at −20°C pending use.

Polymerase chain reaction (PCR) and sequencing

The primers targeting *cox1* of *Pomacea* spp. were PC_F and PC_R of Folmer *et al.* (1994). For samples that failed to be amplified in the PCR, the primers were modified based on previously determined sequences. The modified primers were 5′-AGTTACTTATTCGTGCTG-3′ (PC_F2) and 5′-GTATT AAAATTTCGATCAGT-3′ (PC_R2). PCR cycling conditions used were 94°C for 5 min, and then 35 cycles with 94°C for 60 s, 50°C for 45 s and 72°C for 60 s, followed by 72°C for 10 min for final extension. The PCR products were visualized in a 1% agarose gel and recovered from the gel using mini-spin columns (Axygen). The purified products were used for sequencing by the dideoxynucleotide termination method. The primer used for sequencing was PC_F or PC_F2. All unique mutations were carefully checked, and ambiguous bases were confirmed by resequencing. Sequences were aligned primarily using the Blast web service of NCBI to exclude potential errors or incomplete sequencing. Accurate alignment of the sequences was performed in ClustalX version 2.0 (Larkin *et al.*, 2007).

Data from GenBank

Although the sequences produced in the present study were primarily determined as *P. canaliculata* and *P. insularum* by the Blast web service of NCBI, more accurate identification is still needed. Given the highly complex taxonomy of *Pomacea*, closely related species, including *P. canaliculata*, *P. insularum*, *P. dolioides*, *P. lineata* and *P. paludosa*, were taken into account in our subsequent analysis. The genetic data pertaining to the *cox1* gene of *P. canaliculata*, *P. insularum*, *P. dolioides*, *P. lineata* and *P. paludosa* deposited in GenBank were employed. In total, 244 sequences were available (Table S1), contributed by six studies (Rawlings *et al.*, 2007; Hayes *et al.*, 2008, 2009; Jørgensen *et al.*, 2008; Matsukura *et al.*, 2008; Song *et al.*, 2010). These sequences represented snail samples collected in both native ranges and in areas relatively recently colonized.

Data analysis

The sequences determined in the present study and those from GenBank were completely aligned using ClustalX version 2.0 (Larkin *et al.*, 2007) and trimmed as necessary using BioEdit version 7.0.9.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) for subsequent analyses. The trimmed sequences were then presented to DnaSP version 5.10.01 (Librado & Rozas, 2009) to collapse into unique haplotypes. A network analysis was performed using TCS version 1.21 (Clement *et al.*, 2000) to reveal the relation between haplotypes. The parsimony connection limit was set to 95%, and separate networks were produced. In this network analysis, the frequency of each haplotype from the present study was used to determine the common haplotypes, while the haplotypes from GenBank were set to 1 because of the lack of true frequency.

To deepen our understanding of the phylogenetic placement of the haplotypes identified in the present study, a phylogenetic tree was constructed under Bayesian inference (BI), performed with MrBayes version 3.2.0 (Ronquist & Huelsenbeck, 2003), using all haplotypes from the current study and those from GenBank. Outgroups were chosen according to the rationale of Nixon & Carpenter (1993), that is, using the sister group and dense sampling. Two species, *P. diffusa* (EU528564) and *P. scalaris* (EU528585), were used as outgroups. Both have been introduced to Asia (Hayes *et al.*, 2008), and the '*P. bridgesii* group', to which they belong, is neighbour to the '*P. canaliculata* group' (Hayes *et al.*, 2009). Prior to BI, the best fit nucleotide substitution model (TrN +G) for this dataset was determined using a hierarchical likelihood ratio test in jModeltest version 0.1.1 (Posada, 2008). As the TrN+G model was not an option in MrBayes, an alternative model (GTR+G) was employed for inference. The posterior probabilities were calculated via 11,000,000 generations using Markov chain Monte Carlo (MCMC) simulations, and the chains were sampled every 500 generations. At the end of this run, the average standard deviation of split frequencies was below 0.01, and the potential scale reduction factor was reasonably close to 1.0 for all parameters. A consensus tree was summarized and visualized by Mesquite version 2.75 (Maddison & Maddison, 2011).

The global distribution of haplotypes of *P. canaliculata* and *P. insularum* was mapped using ArcMap version 9.1 by incorporating previous published data (Rawlings *et al.*, 2007; Hayes *et al.*, 2008, 2009; Jørgensen *et al.*, 2008; Matsukura *et al.*, 2008; Song *et al.*, 2010). For the sake of simplicity, localities cited in the literature were collapsed into a single new point when they were in close proximity. Haplotypes that occurred in the original sites were then represented at the newly created point on the map. All haplotypes were grouped according to the results of the network analysis, and their respective proportion in each collection site was visualized as pie charts.

RESULTS

Overall, 521 *cox1* gene sequences of *Pomacea* snails collected from 54 sites were characterized (Table S2). The aligned sequences were trimmed to 503 bp and collapsed into 24 unique haplotypes (Table 1 and Appendix S1). The most common haplotypes were Hap12 and Hap1, accounting for 48.4% and 20.5%, respectively. Less common haplotypes included Hap6 (13.8%), Hap18 (6.5%), Hap5 (4.0%) and Hap21 (2.8%). The remaining haplotypes were rarely found.

The 244 sequences of *P. canaliculata*, *P. insularum*, *P. dolioides*, *P. lineata* and *P. paludosa* obtained from GenBank, together with the 521 sequences from the present study, were collapsed into 112 haplotypes overall. These haplotypes were analysed in MrBayes for inference and creation of a phylogenetic tree (Fig. 1). Most of the haplotypes from the present study clustered with those of *P. canaliculata*, which had been previously identified by

Table 1 Haplotype and frequency of *cox1* gene derived from 544 *Pomacea* spp. collected in 54 sites in P.R. China

Haplotype	Number (%)	Distribution*	Cluster[†]	Species
Hap1	107 (20.5)	33	C	Not confirmed
Hap2	1 (0.2)	1	C	Not confirmed
Hap3	1 (0.2)	1	C	Not confirmed
Hap4	1 (0.2)	1	C	Not confirmed
Hap5	21 (4.0)	4	C	Not confirmed
Hap6	72 (13.8)	23	A	*P. canaliculata*
Hap7	1 (0.2)	1	A	*P. canaliculata*
Hap8	1 (0.2)	1	A	*P. canaliculata*
Hap9	1 (0.2)	1	A	*P. canaliculata*
Hap10	1 (0.2)	1	A	*P. canaliculata*
Hap11	1 (0.2)	1	A	*P. canaliculata*
Hap12	252 (48.4)	41	B	*P. canaliculata*
Hap13	1 (0.2)	1	B	*P. canaliculata*
Hap14	1 (0.2)	1	B	*P. canaliculata*
Hap15	1 (0.2)	1	B	*P. canaliculata*
Hap16	1 (0.2)	1	B	*P. canaliculata*
Hap17	1 (0.2)	1	B	*P. canaliculata*
Hap18	34 (6.5)	12	B	*P. canaliculata*
Hap19	3 (0.6)	2	B	*P. canaliculata*
Hap20	1 (0.2)	1	B	*P. canaliculata*
Hap21	15 (2.8)	6	B	*P. canaliculata*
Hap22	1 (0.2)	1	B	*P. canaliculata*
Hap23	1 (0.2)	1	B	*P. canaliculata*
Hap24	1 (0.2)	1	D	*P. insularum*

*Number of sampling sites.
[†]Categories as referred to in Fig. 1.

Rawlings *et al.* (2007) and Hayes *et al.* (2008), using both morphology and molecular markers. Only one haplotype (Hap24) was clustered with the haplotypes of *P. insularum*. However, clusters C and F were obviously divergent from *P. canaliculata* and *P. insularum*. The genetic distance (Table S3) further supported this observation. Although some haplotypes from these clusters were referred to *P. insularum* and *P. canaliculata* in previous studies (Song *et al.*, 2010), we tentatively consider clusters C and F as separate clades.

Sixteen separate networks consisting of 112 haplotypes were identified (parsimony connection limit of 95%). Only four (networks A–D) contained haplotypes from the present study (Fig. 2). The others (E-P) are indicated in Fig. 1. The majority of specimens in this study fell into networks A, B and C. Only one specimen, identified as Hap24, was included in network D. Cluster C contains six haplotypes, five of them stem from the present study; the sixth haplotype has also been reported from P.R. China (Song *et al.*, 2010). In addition to these four clusters, cluster F contains only one haplotype, which was also discovered in P.R. China (Song *et al.*, 2010).

The geographical distribution of haplotypes within P.R. China showed mosaic-like patterns (Fig. 3). A mixture of several haplotypes at single sites was common. The most widely distributed haplotypes were from clusters B and C, which were found at 46 and 32 of the sites, respectively.

Haplotypes from cluster A were found at 23 sites. The only haplotype in cluster D from the present study was from one site in the Sichuan basin. *P. canaliculata* is the predominant species in P.R. China. Co-occurrence of *P. canaliculata* and *P. insularum* was restricted to only one sampling site in Chongqing.

The global distribution of haplotypes showed a complex pattern. Of 112 haplotypes, only six were found in both native and recently colonized areas. Two of them (exemplified by EU528499 and EU528489) belonged to cluster A (*P. canaliculata*); the former was widely distributed in our study area and other Southeast Asian countries; the latter was only reported from the Philippines and Japan outside its native range (Hayes *et al.*, 2008). The other four haplotypes belong to cluster D (*P. insularum*). Their sequences are represented by GU236487, EF515025, EF515058 and EF514945. Although the haplotype of GU236487 was found in many parts of Southeast Asia, in P.R. China, it had only been reported from the Sichuan basin (Song *et al.*, 2010) and was not identified in the present study. Instead, one close haplotype (Hap24) was discovered in Chongqing municipality, in the Sichuan basin, but represented by only one snail. The remaining three haplotypes were found in the United States (EF515025 and EF515058), Thailand (EF515025), Japan (EF515025) and Korea (EF514945) (Rawlings *et al.*, 2007; Hayes *et al.*, 2008), but have not been found in P.R. China.

Figure 4 shows the global distribution of the 12 haplotype clusters of *P. canaliculata* and *P. insularum*, including clusters C and F, whose specific identities are uncertain. Clusters A, B, D and E were found in both the native and introduced ranges. However, although the same haplotypes did not all occur in both native and introduced ranges, closely related haplotypes in these areas were found in clusters B and E. Clusters C and F were only found in P.R. China. The remaining six clusters (G, H, I, J, K and L), belonging to *P. insularum*, have thus far only been recorded in Brazil.

DISCUSSION

Biological invasions are of growing concern regarding conservation of global biodiversity. Their negative impact on agriculture and food security, ecosystem functioning, and human health and well-being in the invaded areas are well known (Juliano & Lounibos, 2005; Lovell *et al.*, 2006; Olson, 2006; Lv *et al.*, 2008). *P. canaliculata* and related species are typical invasive snails that are now widely distributed across Southeast Asia and the Far East. Thus far, three species of apple snails originating from South America have been confirmed to be introduced into Southeast Asia, namely *P. canaliculata*, *P. insularum* and *P. scalaris* (Hayes *et al.*, 2008). The latter species is restricted to Taiwan, where it probably only occurs in the southern part (Wu *et al.*, 2010). The smaller hatching size and inferior growth performance compared with the other *Pomacea* species might play a role in this regard (Wu *et al.*, 2011). We did not find *P. scalaris* among the more than 500 snail samples collected at 54 sites
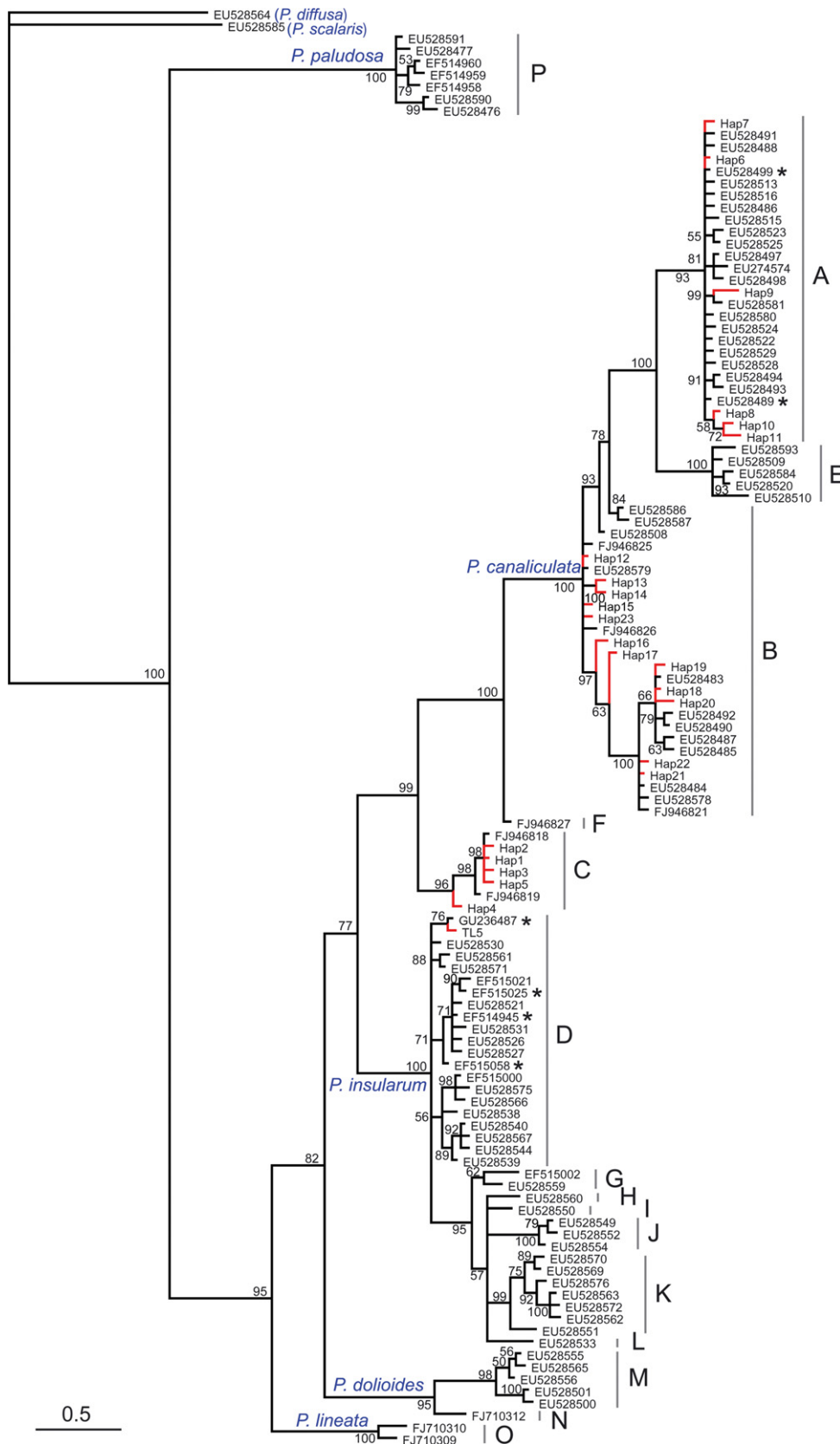
Figure 1 Bayesian inference tree of *Pomacea* haplotypes. The posterior probabilities (percentage) are placed on the branches. The capital letters indicate the haplotype clusters determined by the network analysis. Asterisks denote the haplotypes that occurred in both the native and invaded ranges.
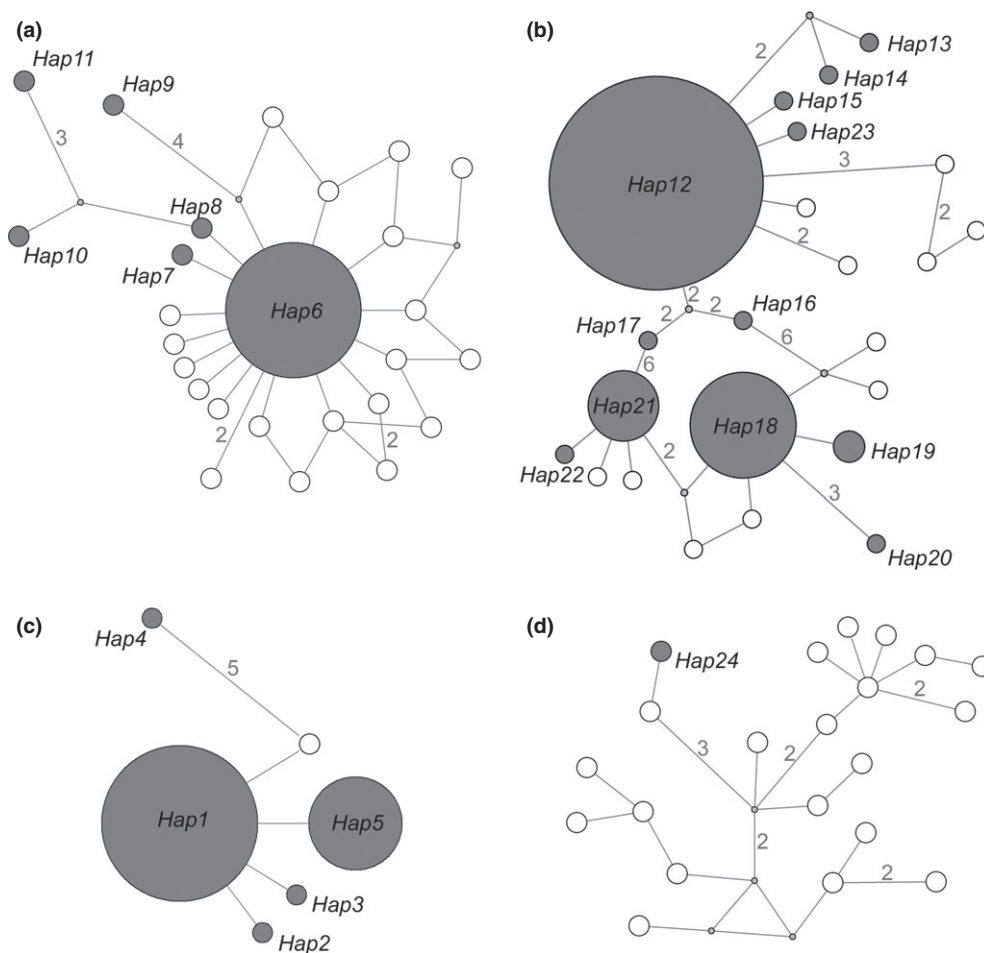
Figure 2 Networks of haplotypes analysed in the present study. The networks are designated A-D, which corresponds to Fig. 1. The number of inferred base substitutions is indicated by the numbers on the connections between haplotypes; connections with no numbers indicate one substitution. The open circles represent haplotypes obtained from previous studies (Rawlings *et al.*, 2007; Hayes *et al.*, 2008, 2009; Jørgensen *et al.*, 2008; Matsukura *et al.*, 2008; Song *et al.*, 2010); filled circles represent haplotypes identified in this study, with their size proportional to frequency.

in P.R. China. In the present study, we identified *P. canaliculata* and *P. insularum* by means of phylogenetic and haplotype network analyses. Our results, along with the findings from other recent investigations, suggest that these two species are most likely to become invasive once introduced. Therefore, any introduction (e.g. traded as pets and food commodities) of such snails should be formally forbidden.

Although molecular approaches confirmed three species of *Pomacea* distributed across Southeast Asia (Hayes *et al.*, 2008), this number of species might be underestimated because of the generally small sample sizes and limited collection efforts, with the exception of sampling in the Philippines, which was part of the study of Hayes *et al.* (2008). In our phylogenetic analysis, we identified two haplotype clusters (C and F), the identity of which is not clear. We did not refer these clusters to particular species for several reasons. First, there was no evidence regarding internal anatomy and shell morphology. Modern taxonomy considers both morphology and internal anatomy as well as molecular informa-

tion (Cowie *et al.*, 2006). Second, no similar sequences in countries other than P.R. China are currently available in the published literature. To date, all *Pomacea* species have not been characterized with molecular markers (*cox1* for the present study). Third, the genetic distance between the clusters and known species (Table S3) suggests species level divergence. Finally, *P. canaliculata* and *P. insularum* are not sister taxa (Hayes *et al.*, 2008, 2009), and thus, it is not impossible that intermediate cryptic species exist. This finding calls for future studies on these clusters.

Introduced populations usually undergo a bottleneck if founded by a few individuals, and genetic variability is expected to decrease in the newly colonized range (Dlugosch & Parker, 2008). Similarly to *P. canaliculata*, the giant African land snail *Achatina fulica* is considered as one of the 100 of the world's worst invasive species (Lowe *et al.*, 2000). Globally, where it has been introduced, *A. fulica* shows rather low diversity, but in its native East Africa exhibits high diversity (Fontanilla, 2010). Following the initial export
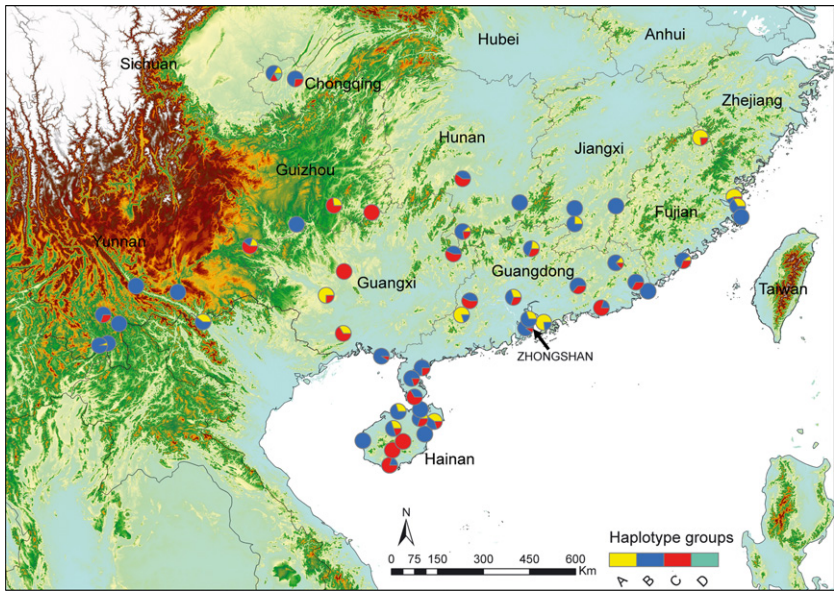
Figure 3 The distribution of haplotypes of *Pomacea* snails in mainland P.R. China. Four groups (A–D) correspond to the groups in Figs 1 & 2. The hatched pies denote *P. insularum*. *P. canaliculata* and group C are indicated by filled pies. The haplotypes from previous studies in P.R. China (Hayes *et al.*, 2008; Song *et al.*, 2010) are not shown. Elevation is shown using different colours.
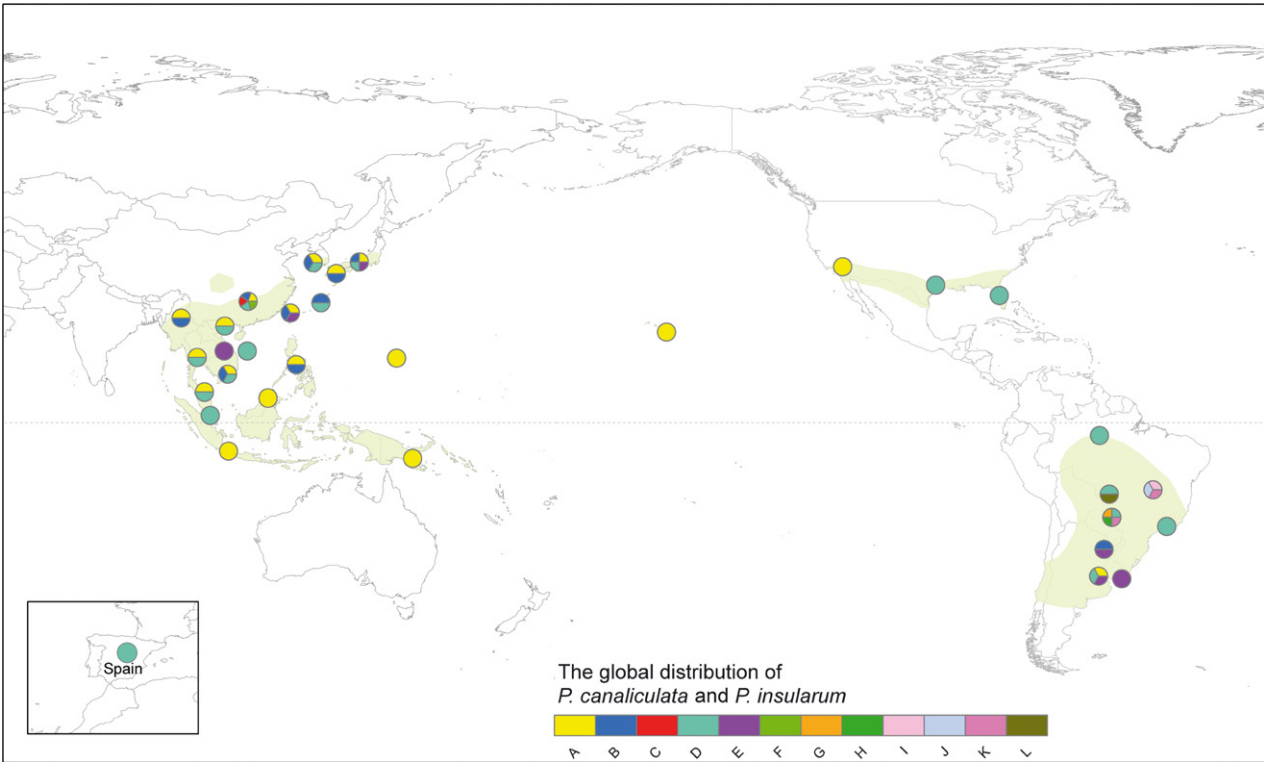

Figure 4 The global distribution of haplotypes of *Pomacea canaliculata* and *P. insularum*. Twelve groups, including groups C and F, which were not specified currently, are indicated using different colours and letters as in Fig. 1. The hatched pies denote *P. insularum* (groups D, G–L). The filled pies indicate *P. canaliculata* (groups A, B and E) and groups C and F. The locations are indicated approximately only, according to previous studies (Rawlings *et al.*, 2007; Hayes *et al.*, 2008; Jørgensen *et al.*, 2008).

of *A. fulica* from East Africa, perhaps to Madagascar (Mead, 1961), secondary introductions (introduction from the invaded range) as a human food commodity led to its subsequent spread across many tropical regions (Mead, 1961). In contrast, the invasion of *Pomacea* snails may follow a different scenario. As shown in the present study, a high

diversity of *Pomacea* haplotypes was found in P.R. China, as has also been demonstrated widely in Southeast Asia (Hayes *et al.*, 2008). Sympatric occurrence of several distinct haplotypes of *Pomacea* spp. at single sites, and different combination of haplotypes among sites, indicate secondary and multiple introductions (Kolbe *et al.*, 2004; Durka *et al.*, 2005; Genton *et al.*, 2005; Lavergne & Molofsky, 2007). For example, according to our results and those of Song *et al.* (2010), in P.R. China, *P. insularum* is restricted to the Sichuan basin, which signals a separate introduction from its native range or from Southeast Asia rather than from the southern part of P.R. China, where *Pomacea* snails were first reported.

Geographical barriers are an important factor governing distribution patterns of native species. For instance, the native amphibious snail species *Oncomelania hupensis*, the only intermediate host of *Schistosoma japonicum* in P.R. China, is highly clustered in different landscapes across large parts of the country (Li *et al.*, 2009a). In contrast, no marked geographical clustering of haplotypes was observed in the present study, although *Pomacea* snails were expected to spread along major water networks. The current mosaic distribution of haplotypes of *Pomacea* snails implies that, rather than natural spread constrained by geographical features, the artificial introductions are the main driver. Indeed, snails from different regions are moved around to areas where they are consumed (Lv *et al.*, 2009a). As a result, local genetic diversity is elevated and thus potentially increases the success of the invasive species (Kolbe *et al.*, 2004; Lavergne & Molofsky, 2007). A recent study predicted that a central region of P.R. China may be susceptible to invasion by these snails in the context of climate change (Lv *et al.*, 2011). Therefore, it is necessary to evaluate the risk of introduction of *Pomacea* in this region and to develop management strategies to prevent establishment and subsequent spread.

In addition to prevention of further intentional introductions, attempts to mitigate the negative impact on crops and biodiversity in native ecosystems should be made. Both *P. canaliculata* and *P. insularum* are extremely polyphagous; they not only consume aquatic macrophytes but also prey on small snails and other aquatic animals (Cazzaniga, 1990; Halwart, 1994; Burlakova *et al.*, 2009; Kwong *et al.*, 2009). The damage to crops in P.R. China is widely acknowledged, and thus, various management measures have been developed to control *Pomacea* snails in rice paddies (Li *et al.*, 2009b). However, the potential impact on native biodiversity is neglected. Nevertheless, studies conducted in other invaded countries, such as Thailand (Carlsson *et al.*, 2004; Carlsson & Brönmark, 2006) and Lao PDR (Carlsson & Lacoursière, 2005) undoubtedly signal similar threats to freshwater communities in P.R. China. Our results provide a genetic basis to discriminate the potential effects of different *Pomacea* species or genotypes on freshwater communities, and hence allow for the development of species- and setting-specific mitigation strategies.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Sequences of haplotypes in FASTA form

Table S1 Available *cox*1 sequences in GenBank

Table S2 Details regarding the 54 sampling locations in the present study

Table S3 Mean genetic distance of haplotype groups

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BIOSKETCH

Shan Lv works on the control of parasite infections and interaction between parasites and hosts. At present, he takes the model of the rat lungworm (*Angiostrongylus cantonensis*) and *Pomacea* spp. to study the impact of invasive species on the parasite infection both in human beings and animals.

Author contributions: S.L., X.N.Z. and J.U. conceived the ideas; S.L., Y.Z., H.X.L. and L.H. collected the specimens; S.L., Q.L., F.R.W., Y.H.G. and W.H. led the laboratory work and data analysis; S.L., P.S., X.N.Z. and J.U. prepared and revised the manuscript.

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