Population genetic patterns of a mangrove-associated frog reveal its colonization history and habitat connectivity

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Abstract

Aim: To understand the impact of historical and contemporary habitat distributions and connectivity on the spatial patterns of a species' genetic variation and divergence, we examined the phylogenetic relationship, population genetic structure and demographic history of a mangrove-specialist, the crab-eating frog (*Fejervarya cancrivora*) from 10 geographic populations in China and northern Vietnam.

Location: Southeast Asian coasts, especially the southern China and northern Vietnam coasts.

Methods: We used the sequences of three mitochondrial DNA (mtDNA) regions to infer phylogenetic relationships and divergence times between our samples and those from other Southeast Asian countries. Thirteen nuclear microsatellite loci were used to analyse population genetic structure. Ancient and more recent demographic history was assessed using mtDNA and microsatellite data, respectively, and population divergence history scenarios were evaluated using approximate Bayesian computation.

Results: The mtDNA haplotypes from China joined the *F. cancrivora* clades from the Philippines, Thailand and Bangladesh, which diverged from the clades from Malaysia and Indonesia. Microsatellite analyses revealed three genetically differentiated clusters and a strong pattern of isolation by distance at the individual level. Ancient population sizes were relatively stable, but genetic signatures of recent population declines were detected. Population divergence history analyses supported that Hainan populations were ancestral to those of the Guangdong, Guangxi and northern Vietnam populations.

Main conclusions: Our results suggest that *F. cancrivora*’s contemporary genetic patterns have been shaped by past and present habitat conformations. This species may have dispersed along the coast from Southeast Asia to China during Pleistocene glaciations when sea levels were low, colonizing the Hainan area first. It subsequently spread to mainland China coasts when sea levels rose and shorelines withdrew. Additionally, current declines and fragmentation of mangrove forests have likely exacerbated population reductions and genetic divergences among now disjunct
1 | INTRODUCTION

Contemporary spatial patterns of a species' genetic diversity are often shaped by a combination of historical and current habitat availability and connectivity. Historical processes such as climate changes, glaciations and palaeogeographic formations may be major drivers of the extant patterns of population genetic diversity (Julio Pineros & Gutierrez-Rodriguez, 2017; Nali et al., 2020; Pinceel et al., 2013; Strugnell et al., 2012). Contemporary factors, including environmental conditions and anthropogenic disturbances, can also strongly impact the geographic distribution of population genetic variation (Cole et al., 2016; Coleman et al., 2018; Wang et al., 2017). Determining how past and present factors contributed to observed genetic structures enables a deeper understanding of species' evolutionary histories and ecological adaptations. It also contributes to the formulation of appropriate conservation and management strategies (McCartney-Melstad & Shaffer, 2015).

Mangrove forests occur in intertidal zones of tropical and subtropical coastlines and provide essential ecosystem services as well as critical habitats for land and marine fauna (Gopal & Chauhan, 2006; Kristensen et al., 2008; Nagelkerken et al., 2008). However, because they are particularly vulnerable to environmental disturbances, sea-level fluctuations and climate change, mangrove forests are rapidly disappearing and now rank as one of the world's most threatened ecosystems (Alongi, 2002; Duke et al., 2007). Additionally, many mangrove-associated species are evolutionarily unique and of importance conservation value (Daru et al., 2013). Conversely, understanding the evolutionary history and population genetic patterns of mangrove-dependent species will help reveal the past distributions and recent conditions of mangrove forests and inform their conservation management.

The crab-eating frog, Fejervarya cancrivora (Gravenhorst, 1829), known for its exceptional adaptation to high salinity habitats throughout all its life stages (Dunson, 1977; Gordon et al., 1961; Uchiyama et al., 1990), is commonly found in tidal mangrove swamps and beaches (Gordon et al., 1961) and is widely distributed in Southeast Asia, including the coastal regions of southern China, Vietnam, Thailand, Bangladesh, Malaysia, Indonesia and the Philippines (Kristensen et al., 2010). Highly sensitive to habitat disturbance, F. cancrivora has been suggested as an indicator species for mangrove habitat conditions (Hong et al., 2011). This frog's unique adaptations make it an ideal species to investigate the dynamics of mangrove forests. However, the taxonomic relationships and evolutionary history of F. cancrivora's various geographic populations remain a subject of debate. For instance, previous studies have suggested the inclusion of several cryptic species within this lineage. Based on allozyme and mitochondrial DNA (mtDNA) evidence, Kurniawan et al. (2010) identified three genetically divergent groups within the currently recognized F. cancrivora species: the mangrove-type from Bangladesh, Thailand and the Philippines; the large-type from Malaysia and Indonesia; and the Pelabuhan ratu/Sulawesi-type (P/S-type) from Indonesia. The mangrove-type shows strict confinement to the mangrove habitat and strong tolerance for salt water, whereas the large-type and the P/S-type do not (Kurniawan et al., 2010). Morphological analyses and crossing experiments confirmed divergences among these groups and supported the proposal that each should be considered a distinct species (Islam et al., 2008; Kurniawan et al., 2010, 2011).

To date, most phylogeographic studies of the species have focused on Southeast Asian countries, whereas the phylogenetic position and evolutionary history of F. cancrivora populations in China remain obscure. A single specimen from Hainan Island in China was grouped with the mangrove-type based on a phylogenetic tree derived from the mitochondrial 16S rRNA gene, and the same study nested a specimen from Taiwan within the large-type clade (Kurniawan et al., 2010). This discrepancy has raised questions both about the phylogenetic position of China’s populations within the species complex and about this species’ historical colonization routes in China. Moreover, no systematic population surveys of the geographic distributions and population statuses of this species in China have been conducted. As mangrove forests are disappearing from southern China’s coastal regions at alarming rate (Chen et al., 2009; Wang & Wang, 2007), F. cancrivora is likely to have experienced population declines and segregations. Yet, there is a lack of knowledge about the impact of mangrove losses on population genetic patterns of this and other mangrove-dependent species.

In this study, we carried out a nationwide population survey and sampling of F. cancrivora in order to resolve these populations’ phylogenetic position within its range and to investigate its population genetic patterns and possible historical and contemporary drivers. To accomplish this, we used a combination of three mtDNA regions and 13 nuclear microsatellite loci of frogs collected from the coasts of southern China and northern Vietnam to reconstruct phylogenetic relationships, assess population genetic structure and infer population demographics and divergence history. In addition to revealing the historical coastal configurations and present mangrove habitat
conditions in China, our results aid conservation planning that can preserve and restore mangroves and their associated biodiversity.

2 | METHODS

2.1 | Field survey and sampling

We searched for *F. cancrivora* along the coastlines of Guangxi, Guangdong and Hainan Provinces between August and September 2013 to 2016, focusing on areas with moderate to large patches of mangrove forests. Those three provinces include most of the tropical coastal regions in China and 94% of all existing mangrove forests in the country (Chen et al., 2009). Field surveys were carried out in the evening (8:00–11:00 p.m.) and researchers with flashlights followed line transects near mangroves and shorelines for about three hours, collecting putative *F. cancrivora* samples. We found the species at nine of 14 localities in the three provinces (Figure 1a) and obtained tissue samples from 79 individuals (Table 1). During our searches, we observed *F. cancrivora* on mudflats adjacent to or inside relatively large and continuous mangrove forests, but never in inland, freshwater environments or far away from mangroves. Additionally, four samples of *F. cancrivora* were collected from a coastal site in Nam Dinh Province of northern Vietnam (Table 1). We were unable to estimate population size at each site due to limited transect surveys and we failed to detect the species at five of the surveyed sites. The mangrove habitats at three of those sites (sites 4, 12 and 13; Table 1; Figure 1a) showed small or highly fragmented forest patches, intensive human disturbance and visible pollutions (e.g. oil slick and accumulated shoreline litter). Mangrove forests at the other two sites (Sites 5 and 14) were in good condition (i.e. large forest areas, continuous distribution and limited anthropogenic modification) and likely contained remnant *F. cancrivora* populations, but stormy weather during the survey period may have thwarted our ability to detect them. We also sampled a number of rice frogs (*Fejervarya limnocharis*) from freshwater ponds and wetlands near the sampling sites. Identities of all samples were later verified using molecular species identification (see below). Tissue samples were preserved individually in 95% ethanol and stored at −20°C for later DNA extraction.

2.2 | Molecular analysis

Total DNA was extracted from tissue samples using the EasyPure Genomic DNA Kit (TransGen Biotech) then DNA quality and concentration were checked by 1% agarose gel electrophoresis and with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.). For each sample, we first determined species identity based on a 490-bp segment of the mtDNA cytochrome b gene (Cytb) sequence (see below). The two morphologically similar species, *F. cancrivora* and *F. limnocharis*, diverged by 19.0% (93 bp) in this sequence.

To find phylogenetic relationships and genetic diversity of *F. cancrivora*, we analysed three mtDNA fragments (Cytb, 16S and D-loop) using primers as described in Table S1, Appendix S1. The designs of

![Figure 1](image-url)
our primers for Cytb and the D-loop region were based on *F. cancrivora* mitochondrial genome sequences (GenBank accession no. EU652694; Ren et al., 2009). We conducted PCR in total volumes of 25 μl, containing approximately 2 ng tissue DNA, 10 μM of each primer, and 12.5 μl 2× EasyTaq PCR SuperMix (TransGen Biotech). The PCR conditions included an initial denaturing at 95°C for 5 min; followed by 35 cycles at 95°C for 30 s, the annealing temperature for each primer pair (see Table S1, Appendix S1) for 30 s and 72°C for 1 min; and a final extension step at 72°C for 10 min. We purified the PCR products using the EasyPure Genomic DNA Kit (TransGen Biotech) and sequenced from both directions on a PRISM 3730 Genetic Analyzer (Applied Biosystems) with the BigDye Cycle Sequencing Kit (Applied Biosystems).

For all samples, we amplified a total of 13 microsatellite loci (A33, A127, B5, B19, B34, B43, B46, B47, B72, B147, B152, C46 and C63) using fluorescent-labelled forward primers and PCR conditions following Zheng et al. (2016). These loci were selected from a panel of variable microsatellite markers developed specifically for *F. cancrivora* in China. We analysed the PCR products on a 3730 XL DNA Analyzer with a GeneScan 500 LIZ size standard (Applied Biosystems) and genotyped them using GENEMAPPER version 3.7 (Applied Biosystems).

### 2.3 Genetic diversity and phylogenetic relationships inferred from mtDNA

We used CLUSTAL W (Larkin et al., 2007) to align mtDNA sequences. Sequences of each mtDNA region were trimmed to identical lengths (Cytb: 557 bp, 16S: 498 bp, D-loop: 763 bp) for subsequent analyses. All unique haplotypes of each mtDNA region were deposited in GenBank (http://www.ncbi.nlm.nih.gov) with accession nos. MK396081-MK396095 (Table S2, Appendix S1). The number of mtDNA haplotypes and polymorphic sites, haplotype diversity and nucleotide diversity were estimated for each region using DNASP version 5.0 (Librado & Rozas, 2009) and the three mtDNA regions (a total length of 1,818 bp) were combined for each individual. Haplotype frequencies were calculated with ARLEQUIN version 3.11.

| Sampling time (Year/Mo) | Site code | Locality | Lat, Long | Distribution | Population code | Sample size |
|-------------------------|-----------|----------|-----------|--------------|-----------------|-------------|
| 2013/09                 | Site 1    | Hainan Province, Haikou, Dongzhaiyang | N19.9523, E110.5931 | Yes | HN1 | 21 |
| 2013/09                 | Site 2    | Hainan Province, Wenchang, Bamenwan | N19.5863, E110.8319 | Yes | HN2 | 12 |
| 2016/08                 | Site 3    | Hainan Province, Danzhou, Xinyinggang | N19.7394, E109.2331 | Yes | HN3 | 5 |
| 2016/08                 | Site 4    | Hainan Province, Chengmai, Wucun | N19.9252, E109.9814 | No | – | 0 |
| 2014/08                 | Site 5    | Guangxi Province, Fangchenggang, Beilunhekou | N21.6649, E108.4154 | Likely | – | 0 |
| 2014/08                 | Site 6    | Guangxi Province, Beihai, Danjiang | N21.6197, E109.0758 | Yes | GX1 | 7 |
| 2014/08                 | Site 7    | Guangxi Province, Beihai, Haicheng | N21.4361, E109.2368 | Yes | GX2 | 1 |
| 2014/08                 | Site 8    | Guangxi Province, Beihai, Shankou | N21.4968, E109.7660 | Yes | GX3 | 14 |
| 2014/08                 | Site 9    | Guangdong Province, Zhanjiang, Gaoqiao | N21.6993, E109.5315 | Yes | GD1 | 6 |
| 2014/08                 | Site 10   | Guangdong Province, Zhanjiang, Taiping | N21.1065, E110.3481 | Yes | GD2 | 6 |
| 2014/08                 | Site 11   | Guangdong Province, Zhanjiang, He’an | N20.6589, E110.3867 | Yes | GD3 | 7 |
| 2014/08                 | Site 12   | Guangdong Province, Zhanjiang, Nanshan | N20.2423, E110.6393 | No | – | 0 |
| 2014/08                 | Site 13   | Guangdong Province, Zhanjiang, Qishui | N20.7561, E109.7603 | No | – | 0 |
| 2014/08                 | Site 14   | Guangdong Province, Zhanjiang, Jiepao | N21.3469 E109.8061 | Likely | – | 0 |
| 2014                 | Site 15   | Vietnam, Nam Dinh Province, Xuan Thuy | N20.2326, E106.5095 | Yes | NQ | 4 |
| Total                  |           |          |           |              |                 | 83 |
(Excoffier et al., 2005; Excoffier & Lischer, 2010). Sequence divergence was evaluated by $p$ distance in MEGA6.0 (Tamura et al., 2013).

We estimated the phylogenetic relationships between different *F. cancrivora* clades using the maximum likelihood (ML) and Bayesian inference (BI) methods based on the Cytb and 16S haplotypes from both China and other Southwest Asian countries (see Table S2, Appendix S1). Corresponding *F. cancrivora* D-loop sequences from other countries were unavailable for this analysis. The corresponding sequences from *Fejervarya iskandari* (GenBank acc. nos. AB296085 and AB277303) and *F. limnocharis* (GenBank acc. nos. MK411008 and AB070737) were used as outgroups. Using MAFFT version 7 online (Katoh et al., 2019), we aligned the sequences and then further trimmed our Cytb sequences to 490 bp and our 16S sequences to 392 bp, sequence lengths identical to those from other studies. The Cytb and 16S datasets were concatenated (Cytb + 16S) using MEGA 6 (Tamura et al., 2013) and missing bases were filled withNs. Optimal nucleotide substitution models and partition setting were determined using PARTITIONFINDER version 2.1.1 (Guindon et al., 2010; Lanfear et al., 2017) and the corrected Akaile information criterion. The ML tree was constructed in IQ-TREE version 1.6.2 (Lam-Tung et al., 2015) with 1,000 bootstrap replicates. BI analysis was performed in MRBAYES version 3.2.6 (Ronquist et al., 2012) using default priors for three independent runs with one cold chain and three heated chains in each run. Markov chain Monte Carlo (MCMC) was ran for 10 million generations and sampled every 1,000 generations with a burn-in set to 25%. We used TRACER version 1.7 (Rambaut et al., 2018) to check the result for chain convergence and posterior sample size.

In addition, we estimated the divergence times between different *F. cancrivora* clades with BEAST version 2.6.3 (Bouckaert et al., 2019) using the Cytb + 16S haplotypes. Due to the lack of fossil-based calibration points, we employed a lognormal relaxed clock (uncorrelated), an average mtDNA substitution rate in anurans of 1% per million years (Yan et al., 2013; Zhang et al., 2010) and an average clock (uncorrelated), an average mtDNA substitution rate in anurans of 1% per million years. Optimal nucleotide substitution models and partition setting were determined using PARTITIONFINDER version 2.1.1 (Guindon et al., 2010; Lanfear et al., 2017) and the corrected Akaile information criterion. The ML tree was constructed in IQ-TREE version 1.6.2 (Lam-Tung et al., 2015) with 1,000 bootstrap replicates. BI analysis was performed in MRBAYES version 3.2.6 (Ronquist et al., 2012) using default priors for three independent runs with one cold chain and three heated chains in each run. Markov chain Monte Carlo (MCMC) was ran for 10 million generations and sampled every 1,000 generations with a burn-in set to 25%. We used TRACER version 1.7 (Rambaut et al., 2018) to check the result for chain convergence and posterior sample size. We required an effective sampling size of more than 200 for all parameters.

To better visualize phylogenetic relationships among *F. cancrivora* populations sampled in this study, we constructed a haplotype network for each of the three mtDNA regions and for the concatenated sequences (a total length of 1,818 bp) of each sample using the median-joining method in NETWORK version 5.0 (Bandelt et al., 1999).

### 2.4 Genetic diversity and population structure inferred from microsatellite data

For the microsatellite data, we used MICROCHECKER version 2.2.3 (van Oosterhout et al., 2004) to estimate the occurrences of allelic dropout, null alleles and scoring errors. Hardy-Weinberg equilibrium and linkage disequilibrium between loci were evaluated in GENEPOP version 3.4 (Raymond & Rousset, 1995), and for multiple comparisons, $p$ values were adjusted using the Bonferroni correction (Rice, 1989). We quantified the genetic diversity of the microsatellite loci in each sampled population by using the following estimates: the observed and expected heterozygosity and polymorphism information content, calculated in CERVUS version 3.0.3 (Kalinowski et al., 2007); allelic richness, calculated in HP-RARE version 1.0 (Kalinowski, 2005); and the inbreeding coefficient $F_{IS}$, calculated in FSTAT version 2.9.3.2 (Goudet, 1995).

Isolation by distance (IBD) refers to a positive correlation of genetic and geographic distance that is often observed in species with spatially limited gene flow (Rousset, 1997; Wright, 1943). We used Mantel tests implemented in GENALEX version 6.5 (Peakall & Smouse, 2006, 2012) to assess the correlation between pairwise Nei’s genetic distance (calculated with GENALEX) and geographic distance among sampled individuals. Geographic distance among samples was measured as the natural logarithm of the present coastline distance. As *F. cancrivora* is closely associated with coastal mangrove habitats, coastline distance is a more biologically realistic measure of dispersal than is straight-line distance (Karns et al., 2000). Present coastline distances between our sampling locations were obtained using Google Earth (http://www.google.com/earth/) and the open ocean between Hainan Island and the Leizhou Peninsula was measured as the shortest distance across the Qiongzhou Strait. Statistical significance was evaluated by comparing the estimated $r$ to a distribution of $r$-scores calculated with 10,000 permutations.

We used two methods to infer population genetic structure based on our samples’ microsatellite data. First, we used a Bayesian clustering algorithm in STRUCTURE version 2.3.4 (Pritchard et al., 2000) in which the value of the number of genetic clusters, $K$, was set to 1–10, with the largest $K$ equal to the number of sampled localities. Ten independent runs were performed for each $K$ value, using the admixture model with correlated allele frequencies among clusters. Simulations were run for one million MCMC iterations after a burn-in of 100,000. The most likely $K$ value was determined using the $\Delta K$ method following Evanno et al. (2005), as implemented in STRUCTURE HARVESTER version 0.6.94 (Earl & vonHoldt, 2012). To detect the potential hierarchical population structure nested within the first level of genetic clustering, we repeated an iterated STRUCTURE analysis on each identified cluster until the genetic cluster was inferred down to one (Evanno et al., 2005; Janes et al., 2017). The STRUCTURE results for the most likely value of $K$ were summarized using CLUMPP version 1.1 (Jakobsson & Rosenberg, 2007) with 1,000 permutations and the FullSearch algorithm and plotted using DISTRUCT version 1.1 (Rosenberg, 2004).

We also performed principal coordinate analysis (PCoA) using GENALEX to assess population genetic structure based on genotypic genetic distances between individuals.

Estimates of the effective number of migrants ($N_m$) among the inferred clusters were assessed using a Bayesian coalescent-based
framework under the default setting in MIGRATE-N version 3.6.11 (Beerli, 2006; Beerli & Palczewski, 2010). MIGRATE-N provides estimates of $\theta$ ($4N_e\mu$; $N_e$ = effective population size, $\mu$ = mutation rate) and $M$ ($m/\mu$; $m$ = migration rate) from microsatellite data. We ran five independent replications for each genetic cluster under the Brownian stepwise mutation model with initial parameters based on $F_{ST}$ values and constant mutation rates. We conducted searches using 5 million steps for each replication, with 500,000 discarded as burn-in, and sampled the parameter values every 20 iterations. We calculated $N_m$ using the equation $N_m = (\theta \times M)/4$.

FIGURE 2 Six likely scenarios of population divergence in the DIYABC analysis using Fejervarya cancrivora microsatellite data. Each scenario represents a divergence order among the three genetic clusters inferred from microsatellite data. Time is not strictly to scale. See Figure 1 for map labels.
2.5 | Demographic history and population divergence

The different mutation rates of mtDNA and microsatellite loci allow inferences of population demographic patterns in different time frames. Slowly evolving mtDNA can reveal ancient population demographic history, whereas the rapid mutation rates of microsatellites enable tests of ancient as well as recent demographic events (Fumey et al., 2018; Zhang et al., 2014). We evaluated the ancient historical demographic changes of the inferred genetic clusters using the combined mtDNA sequence (1,818 bp) data. Because genetic Clusters 2 and 3 each contained few mtDNA haplotypes, we combined the data of those two clusters for the subsequent analyses.

First, we estimated Tajima’s D (Tajima, 1989) and Fu’s Fₜ statistics (Fu, 1997) in DNASP to seek the genetic signature of demographic expansions. We then performed Bayesian skyline plots with BEAST to assess ancient demographic dynamics in the study populations. This analysis was conducted using the same settings as above for divergence time estimation. The data failed to generate an effective sample size (n > 200) when Clusters 2 and 3 were analysed both separately and combined, likely because of the limited sample sizes of those populations. Therefore, we conducted this analysis only on data from Cluster 1 and the total population.

We used our microsatellite data to analyse population divergence history through approximate Bayesian computation (ABC) implemented in DIYABC version 2.0.1 (Cornuet et al., 2008, 2014). Since both the statistical inference power and the accuracy of parameter estimation increase with both reduced model complexity and the number of competing models using DIYABC (Cabrera & Palsbøll, 2017), we focused on the major historical population divergence events and did not model demographic changes in recent time frames in the ABC scenarios. Because statistical inferences using mtDNA detected no large historical population expansion or decline and because the results of population structure analyses showed significant genetic differentiations and low migration rates between the inferred genetic clusters (see Section 3), we did not consider population size changes or gene flow between clusters in our population divergence history simulations. Due to the species’ close association with mangrove habitats, we assumed that its colonization from Southeast Asia to China occurred linearly along the coastline. Therefore, we defined six biologically meaningful scenarios of historical divergence for the three genetic clusters (Figure 2a). Scenario 1 was the null model where all three clusters split simultaneously from an ancestral population at t₁. The other five scenarios considered sequential divergences between the inferred genetic clusters. Scenario 2 considered Cluster 3 as the first population colonizing the coasts of southern China and from which Cluster 2 diverged at t₂, followed by divergence of Cluster 1 from Cluster 2 at t₃ (i.e. a dispersal route from Guangxi to Guangdong, then to Hainan). Scenario 3 considered Cluster 2 as the first population colonizing the coasts of China and from which first Cluster 1 and then Cluster 3 diverged at t₂ and t₃, respectively (i.e. a dispersal route from Guangdong to Hainan and Guangxi). Scenarios 4 through 6 all considered Cluster 1 as the first population colonizing the coasts of China. In Scenario 4, Cluster 2 diverged from Cluster 1 at t₂ and Cluster 3 split from Cluster 2 at t₃ (i.e. a dispersal route from Hainan to Guangdong, then to Guangxi). In Scenario 5, Cluster 3 diverged first from Cluster 1 at t₂ and then Cluster 2 split from Cluster 3 at t₃ (i.e. a dispersal route from Hainan to Guangxi, then to Guangdong). Finally, in Scenario 6, a population diverged from Cluster 1 at t₂ and subsequently split to Clusters 2 and 3 at t₃ (i.e. a population diverged from the Hainan population and split to the Guangdong and Guangxi populations) (Figure 2a). We set prior values for Nₑ and divergence time estimates with a uniform distribution for all parameters (Table S3, Appendix S1). In all scenarios, we assumed that each of the three clusters had a constant size over time and post-divergence from the ancestral population. The microsatellite mutation rate was set between 1 × 10⁻⁵ and 1 × 10⁻⁴ substitutions/generation (Komaki et al., 2017) with a uniform distribution and under the stepwise mutation model. All loci were set to 4-bp repeats except A33 and A127, which were set to 2-bp repeats (Zheng et al., 2016). We used default settings for the other microsatellite parameters. We simulated six million datasets for each scenario and calculated summary statistics (mean number of alleles per locus, mean genetic diversity and mean Garza–Williamson’s M) for each cluster and Fₛₜ and the mean classification index between pairs of clusters. We estimated the posterior probabilities of the modelled scenarios using a logistic regression plot of the 1% of simulated datasets closest to the observed data (Cornuet et al., 2008). The most likely scenario was the one with the highest significant posterior probability value and non-overlapping 95% confidence interval (CI). For this scenario, we estimated type I (probability of rejecting the selected scenario when it is true) and type II (probability of selecting the scenario when it is false) errors by using the “confidence in scenario choice” function in DIYABC with 1,000 independent datasets and logistic regression approaches (Cornuet et al., 2010). We also used principal component analysis in DIYABC to check goodness-of-fit between the simulated and observed data by using 10,000 simulations of the most likely scenario.

Finally, we used the microsatellite data with BOTTLENECK version 1.2.02 (Cornuet & Luikart, 1996; Piry et al., 1999) to detect the molecular signatures of recent population declines. We conducted the analysis under both the stepwise (SMM) and the two-phased mutation models (TPM), with 95% single-step mutations and 5% multi-step mutations. We tested for the significance of bottleneck signatures using the sign test and Wilcoxon’s signed-rank test.

3 | RESULTS

3.1 | Genetic diversity and phylogenetic relationships inferred from mtDNA

Three mtDNA fragments (Cytb, 16S and the D-loop region) were successfully sequenced for all 83 samples. Six, five and four unique haplotypes were recovered from the Cytb, 16S and D-loop sequences, respectively, and combining the three fragments from each
individual yielded nine haplotypes (ChMT-1–ChMT-9). The number of haplotypes and diversity estimates for each sampling locality are shown in Table 2.

We constructed phylogenetic trees using Cytb and 16S sequences from our samples and from *F. cancrivora* samples from other Southeast Asian countries (Figure 3). Both ML and BI trees showed similar topologies (Figure S1, Appendix S1). All haplotypes from our study grouped with the mangrove-type haplotypes from the Philippines, Thailand, India and Bangladesh. We estimated the divergence event between the mangrove-type and the clade of the large-type from Malaysia and Indonesia and the P/S-type from Indonesia to be 9.95 Mya (95% highest posterior density [HPD]: 4.23–8.44). Sequence divergences estimated by mtDNA haplotypes. The other four haplotypes (ChMT-3, ChMT-6, ChMT-8 and ChMT-9) differed from ChMT-4 at 7–14 sites, suggesting a relatively deeper divergence history for those haplotypes.

### 3.2 Genetic diversity and population structure inferred from microsatellite data

Samples from 83 frogs collected at 10 localities were successfully PCR-amplified and genotyped. Seventy-three (88%) samples were genotyped at 12–13 microsatellite loci, while 10 lacked genotype data at two to three loci. The number of alleles varied from 5 to 27, with a mean of 13 (SD 6.3) alleles per locus (Table S4, Appendix S1). Genetic diversity estimates for each sampled population are shown in Table 2. Three, two and zero loci showed significant departure from Hardy–Weinberg equilibrium in the inferred genetic Clusters 1, 2 and 3 (see below), respectively, while no linkage disequilibrium was found between any loci. Two loci showed evidence of null alleles in Cluster 1, but estimated percentages of null alleles were low (<8%). A greater number of loci showed null alleles in Clusters 2 and 3, but this pattern was likely due to small sample sizes so we retained those loci in the following analyses.

### TABLE 2 Summary of genetic diversity parameters of the mtDNA (combined sequences of 1,818 bp) and 13 microsatellite loci of *Fejervarya cancrivora* sampled from 10 localities in China and northern Vietnam

| Population | N | n | h (SD) | π (SD) | Unique Haplotype | A | A_R | H_O | H_E | PIC | F_IS |
|------------|---|---|--------|--------|-----------------|---|-----|------|------|-----|------|
| HN1        | 21 | 5 | 0.748 (0.064) | 0.0027 (0.0027) | ChMT-1,2-5 | 7.6 | 6.18 | 0.747 | 0.746 | 0.689 | -0.001 |
| HN2        | 12 | 1 | 0     | 0      | ChMT-9 | 5.1 | 4.10 | 0.593 | 0.626 | 0.556 | 0.055 |
| HN3        | 5  | 1 | 0     | 0      | ChMT-6 | 4.4 | 3.15 | –     | –     | –    | –    |
| GX1        | 7  | 1 | 0     | 0      | –     | 4.2 | 3.40 | 0.546 | 0.639 | 0.551 | 0.158 |
| GX2        | 1  | 1 | 0     | 0      | –     | 1.3 | 0.85 | –     | –     | –    | –    |
| GX3        | 14 | 2 | 0.143 (0.119) | 0.0001 (0.0001) | ChMT-7 | 6.3 | 5.19 | 0.634 | 0.726 | 0.663 | 0.132 |
| GD1        | 6  | 1 | 0     | 0      | –     | 4.5 | 3.18 | –     | –     | –    | –    |
| GD2        | 6  | 2 | 0.333 (0.215) | 0.0024 (0.0015) | –     | 3.9 | 3.04 | –     | –     | –    | –    |
| GD3        | 7  | 3 | 0.524 (0.209) | 0.0022 (0.0014) | ChMT-8 | 3.6 | 3.01 | 0.571 | 0.592 | 0.492 | 0.040 |
| NQ         | 4  | 1 | 0     | 0      | –     | 4.4 | 2.88 | –     | –     | –    | –    |
| Total      | 83 | 9 | 0.657 (0.051) | 0.0032 (0.0004) | –     | 13.0 | 11.44 | 0.657 | 0.813 | 0.785 | 0.190 |

Note: Genetic diversity parameters of microsatellite data were calculated only for populations with seven or more samples. For mtDNA data: N, sample size; n, number of haplotypes; h, haplotype diversity; π, nucleotide diversity; SD, standard deviation; Unique Hap, unique haplotype(s) in each sampling locality. For microsatellite data: A, mean number of alleles per locus; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content; F_IS, inbreeding coefficient.
The Mantel test of microsatellite data revealed a significant positive correlation between pairwise genetic distance and geographic distance (coastline distance) among sampled individuals (Figure 4), indicating a strong pattern of IBD.

**FIGURE 3** Phylogenetic relationships and divergence time estimates of *Fejervarya cancrivora* haplotypes using an 882 bp (Cytb 490 bp + 16S 392 bp) mitochondrial sequence generated in BEAST. Detailed information for the haplotypes are shown in Table S2, Appendix S1. Numbers above the nodes indicate medians of the estimated divergence time (in Mya) and blue bars show the 95% highest posterior density intervals. Bootstrap values for maximum-likelihood analysis/Bayesian posterior probability are shown under the nodes.

**FIGURE 4** Correlation between pairwise genetic distances and geographic distances among 83 individuals. A positive relationship was found between Nei's genetic distances and geographic distances (km) along the coastlines (Mantel test: $r = .470$, $p < .001$)

STRUCTURE analysis showed that the number of clusters suggested by the calculation of $\Delta K$ values was $K = 2$, implying the likely presence of two genetically distinct groups (Figure 5a and Figure S3a, Appendix S1). All samples from Hainan Province (HN1, HN2 and HN3) and the east coast of the Leizhou Peninsula in Guangdong Province (GD2 and GD3) formed one cluster (Cluster 1), despite the presence of the Qiongzhou Strait between Hainan Island and the Leizhou Peninsula, and all the other samples formed a second cluster. Subsequent hierarchical STRUCTURE analyses of the inferred clusters showed that the second cluster could be further divided into two genetic groups. Samples from GD1, GX2, GX3 and NQ grouped together (Cluster 2), whereas samples from GX1 formed another group (Cluster 3) (Figure 5a and Figure S3b, Appendix S1; see also Figure 1a). No further genetic structure was detected within Clusters 1, 2 and 3. Boundaries between the inferred clusters were discrete, indicating that genetic isolation may have already formed between clusters.

Like the STRUCTURE clustering results, the individual-based PCoA plot revealed three main genetic groups (Figure 5b). The first axis described 39% of the variation in the microsatellite data and clearly differentiated Cluster 1, inferred in STRUCTURE analysis, from the rest of samples. Samples constituting Cluster 1 (all HN samples, GD2 and GD3) formed the most genetically distinct group.
Clusters 2 and 3 were also genetically differentiated along the second axis, although to a lesser extent compared to that between Cluster 1 and the other samples (Figure 5b).

Genetic divergences among clusters defined by population structure analyses were also revealed by $F_{ST}$ analysis and migration rate estimates using microsatellite data. Values of mean inter-cluster $F_{ST}$ ranged from 0.155 (between Clusters 2 and 3) to 0.194 (between Clusters 1 and 2) to 0.300 (between Clusters 1 and 3), and all pairwise comparisons were statistically significant (all $p < .001$), thus suggesting strong genetic differentiation among all clusters. With the largest pairwise $F_{ST}$ values, Cluster 1 was the most genetically diverged cluster.

MIGRATE analysis showed migration rates ($N_{m}$) ranging from 0.1 (from Cluster 3 to 1) to 0.9 (from Cluster 3 to 2) individuals per generation (Table 3), thus indicating low levels of gene flow between all cluster pairs.

3.3 | Demographic history and population divergence

We inferred the ancient population demographic history using the combined mtDNA sequences in neutrality tests and Bayesian skyline plots. Tajima’s $D$ and Fu’s $F_{S}$ statistics were positive in Cluster
Table 3: Bidirectional estimates of gene flow between the inferred genetic clusters as shown by migration rates (effective number of migrants per generation) calculated in MIGRATE using microsatellite data

| Migrate from | Cluster 1 | Cluster 2 | Cluster 3 |
|--------------|-----------|-----------|-----------|
| Cluster 1    | 0.207     | 0.101     |           |
| Cluster 2    | 0.448     |           | 0.913     |
| Cluster 3    | 0.862     | 0.700     |           |

Table 4: Summary of BOTTLENECK tests using the microsatellite data of the inferred genetic clusters

| Cluster | Sign test | Wilcoxon’s signed-rank test |
|---------|-----------|-----------------------------|
|         | SMM       | TPM                         | SMM       | TPM     |
| Cluster 1 | 0.002     | 0.002                       | 0.001     | 0.002   |
| Cluster 2 | 0.044     | 0.041                       | 0.007     | 0.080   |
| Cluster 3 | 0.350     | 0.368                       | 0.735     | 0.635   |
| All       | 0.001     | 0.108                       | 0.004     | 0.068   |

Note: Values in bold type are statistically significant (p < 0.05).

Abbreviations: SMM, stepwise mutation model; TPM, two-phased mutation model.

1 and in the total population but negative in the combined Cluster 2–3 (non-significant p values for most of the examined clades, except for Fu’s F_1 for Cluster 2–3; Table S5, Appendix S1). This suggests a historical lack of large population expansion or contraction. The Bayesian skyline plots showed overall stable historical effective population sizes for Cluster 1 and for the total population (Figure S4, Appendix S1).

The most supported scenario of F. cancrivora population divergence history, using DIYABC analysis, was Scenario 6 (Figure 2 and Figure S5, Appendix S1), in which a first historical divergence event occurred between Cluster 1 and the other populations, and a second event occurred with the divergence of Clusters 2 and 3 from a common ancestral population (logistic approach PP = 0.605, 95% CI = 0.584–0.627). The posterior probability of Scenario 6 was considerably higher than that of Scenario 4 (PP = 0.250, 95% CI = 0.218–0.283), the second highest ranked scenario and that of the other scenarios, which varied between 0.000 and 0.140 (Table S6, Appendix S1). Scenario 6 showed high type I (0.525) but generally low type II errors (mean distribution of 0.000 and 0.140). This supports that F. cancrivora that is currently distributed on the Chinese Peninsula, followed by a secondary and more recent divergence that separated Clusters 2 and 3. Analysis of mtDNA sequences also showed that Cluster 1 had a much greater number of unique haplotypes than the other populations (Table 2), a result consistent with the expectation for ancestral populations. Based on this geographic

4 | DISCUSSION

4.1 | Phylogenetic relationships and colonization history of Chinese Fejervarya cancrivora

The phylogenetic relationships and taxonomic structure among species of the genus Fejervarya have long been a topic of confusion. Species identification based on morphological characters is often problematic in this clade, owing to minor interspecific differences and intraspecific morphological variations, and because morphologically close groups may show considerable genetic divergence and reproductive isolation (Islam et al., 2008; Kotaki et al., 2010). Molecular phylogenetic analyses have revealed many cryptic species and erroneous species identifications (Djong et al., 2007; Sumida et al., 2007; Toda et al., 1998). This all points to a need for careful examination and validation of phylogenetic relationships in the currently recognized genus Fejervarya. Our mtDNA analysis of the F. cancrivora sampled in China and Vietnam provides the first molecular evidence of the phylogenetic position of these populations within the species complex. Our results placed our samples within the mangrove-type also found in Bangladesh, Thailand and the Philippines, which diverged from the large- and P/S-type clades. The deep divergence history and large mtDNA sequence divergences among the mangrove-, large- and P/S-type support that F. cancrivora is a complex of cryptic species and the three types can be considered three distinct species (Kurniawan et al., 2010, 2011).

The limited sequence divergences and phylogenetic relationships between our samples and those from the Philippines, Thailand and Bangladesh suggest that the expansion of this species from Southeast Asia to China occurred relatively late. It is known that repeated glaciations during the Pleistocene caused sea levels to drop below the present level for extended periods of time (Voris, 2000; Yao et al., 2009). At low sea levels, the Beibu Gulf area was exposed above sea level and southern Vietnam and the Hainan area were directly connected by a short coastline (Voris, 2000) (Figure 6). Furthermore, population divergence history inferred using the ABC approach supports the Hainan population (Cluster 1) as the ancestral population of the F. cancrivora that is currently distributed on the coasts of China and northern Vietnam. A lineage of this cluster may have diverged to give rise to populations west of the Leizhou Peninsula, followed by a secondary and more recent divergence that separated Clusters 2 and 3. Analysis of mtDNA sequences also showed that Cluster 1 had a much greater number of unique haplotypes than the other populations (Table 2), a result consistent with the expectation for ancestral populations. Based on this geographic
and genetic evidence, we speculate a history of *F. cancrivora* population expansion and colonization in China (Figure 6). First, during Pleistocene glaciations the species dispersed along this coastline from Southeast Asia to colonize Hainan, which may have served as a refuge for the species when sea level rose and flooded the surrounding landmass. As coastlines of continental China retreated, the frog subsequently moved across the Qiongzhou Strait, possibly when there was still land connecting the Leizhou Peninsula and Hainan Island, and then, it dispersed northward along both sides of the peninsula. The population on the west coast of the peninsula continued to move westward along the coastline, colonizing the Guangdong, Guangxi and northern Vietnam coasts. This hypothetical dispersal pattern is in stark contrast to the intuitive notion, based on contemporary coastal configurations, that the species spread along the Indochinese peninsular coasts from Southeast Asia to southern and then northern Vietnam, and reached its current ranges in China, first in Guangxi and then Guangdong, and lastly, Hainan. Interestingly, molecular genetic studies of mangrove species on the coast of Vietnam also showed significant genetic differentiation between the northern and southern populations, supporting the presence of gene flow barriers along the coast (Guo et al., 2018; Kado et al., 2004). However, the high type I error for the most supported population divergence scenario suggests limited confidence of the ABC analysis, and we cannot definitively prove our dispersal route hypothesis due to the limited geographic scope of our samples. Mangrove-type *F. cancrivora* samples from other Southeast Asian countries (e.g., India, Bangladesh, Thailand, southern Vietnam and the Philippines) will help resolve the intraspecific relationships and evolutionary history of this species.

An interesting question remains regarding the origin and phylogenetic position of the *F. cancrivora* population on the island of Taiwan. The 16S rRNA gene sequence of a specimen from southern Taiwan grouped with the large-type haplotypes from Indonesia and Malaysia, but was separate from the mangrove-type (Kurniawan et al., 2010). Samples from the landmass close to Taiwan, including mainland China to its west and the Philippines to its south, were all shown to be the mangrove type (Kurniawan et al., 2010; this study). It is likely that the Taiwan population may have been introduced from Indonesia or Malaysia, possibly on a ship travelling between ports, thus explaining its different origin from the other populations.

**FIGURE 6** Map of the sea coast contours at 100 m below present sea level. Arrows indicate hypothetical *Fejervarya cancrivora* dispersal routes based on population genetic analyses: first from Southeast Asia to Hainan, China, and subsequently northward and westward along the China coast when sea levels rose and coastlines retreated. The species’ westward expansion eventually reached northern Vietnam. The map of sea level contours was redrawn from Voris (2000)
in southern China. Similarly, an introduced *F. cancrivora* population, transported from East Asia, was also reported in Guam (Christy et al., 2007).

### 4.2 Population genetic structure of *Fejervarya cancrivora* in China

A strong IBD pattern of genetic differentiation was detected among all sampled populations in southern China, thus supporting the likelihood that the species may have a limited capacity for long-distance movement and that most dispersal may occur in small incremental steps between adjacent populations. Similar patterns of IBD were also reported in a coastal mangrove-associated snake (Karns et al., 2000), thus suggesting a commonality among mangrove-dependent species in their dispersal modes, which are possibly shaped by the patchily distributed habitat along coastlines.

Genetic analysis of the population structure using the microsatellite data revealed three genetically diverged groups among our sampled populations. All populations in Hainan and east of the Leizhou Peninsula clustered into one of the groups (Cluster 1), suggesting that there had been relatively high levels of genetic exchange among those populations, at least until recent times. Our field surveys found that mangrove forests in the northern coast of Hainan Island and on the east coast of the Leizhou Peninsula were overall in better conditions (e.g., coverage size, forest maturity and anthropogenic degradation) than those in many other regions, such as the west coast of the Leizhou Peninsula and the Guangxi Province coast. This healthy and continuous habitat likely supports relatively abundant *F. cancrivora* populations and adequate gene flow between geographic groups along the same coastline. Genetic evidence also suggests a lack of gene flow barriers between the Hainan and east Leizhou populations, despite the Qiongzhou Strait, which is about 19 km wide at the narrowest point with a maximum depth of 114 m below sea level. Paleoecological analyses indicated that the Qiongzhou Strait was exposed above sea level for most of the latest glacial period and only opened up at about 8.5 ka cal BP, reaching its current coastline was exposed above sea level for most of the latest glacial period and at the narrowest point with a maximum depth of 114 m below sea level. Mangrove forests along the west coast of the Leizhou Peninsula are small with a very patchy distribution. Shore geomorphology, estuarine formation and climate conditions may partly explain the restricted mangrove growth, but human deforestation and pollution have clearly aggravated the situation by reducing the size and connectivity of mangroves in this region. Gene flow by natural dispersal between Clusters 1 and 2 would be unlikely under the present conditions unless there are unidentified remnant populations along the west coast of the Leizhou Peninsula, and genetic exchange among the distant groups can be achieved in a step-wise manner between geographically close populations. Clusters 2 and 3 showed a slightly greater level of gene flow but bidirectional *N_m* estimates were still less than one, suggesting the presence of dispersal barriers between the two clusters, despite relatively short coastline distances. Interestingly, the one Vietnam population was genetically grouped with Cluster 2 despite the long geographic distance, thus suggesting frequent inter-population gene flow that may be maintained by either ocean currents in the Beibu Gulf (Daryabor et al., 2016; Gao et al., 2013) or human-mediated transport from Guangxi to Vietnam.

### 4.3 Conservation implications for mangrove-dependent biota

Southeast Asia holds most of the world’s mangrove forests (Giri et al., 2011), yet mangroves are rapidly disappearing due to that region’s urban and economic developmental pressures (Duke et al., 2007; Lovelock et al., 2015). From the 1950s to 2000, Chinese mangrove forests have been reduced by almost 50% (Dan et al., 2016). We detected strong genetic signatures of recent population bottlenecks that correlate with documented areal declines of mangrove forests in the last century. Mangrove recovery and restoration campaigns during the last few decades have significantly increased forest ranges in some regions (Dan et al., 2016), but mangrove deforestation and deterioration are still common at local levels. In fact, at Wucun in Hainan Province (Site 4, Table 1), an *F. cancrivora* population found in 2008 (Hong et al., 2011) was no longer detectable in 2016 by the same researchers, possibly a result of severe mangrove reduction and aquaculture expansion at this site during the intervening years. We expect that many local populations of *F. cancrivora* have declined greatly or have been extirpated along the Chinese coasts because of habitat destruction, water pollution and frog harvesting in the last few decades.

Mangrove forests provide essential habitats for highly diverse terrestrial and marine biota, and the rapid disappearance of this critical habitat is inevitably threatening its dependent biodiversity (Daru et al., 2013; Polidoro et al., 2010). However, very few studies have investigated the impact of mangrove loss on the population genetic structure of associated species. The strong genetic differentiations among population clusters of *F. cancrivora* may have roots in historical climatic and geographic processes, but the recent and ongoing areal declines of mangrove forests are likely aggravating the genetic isolation among local populations. Further population surveys and genetic monitoring of this and other mangrove-associated species should be carried out to evaluate the effects of mangrove deforestation and restoration on dependent biota.

Based on the results of our genetic analyses and field survey, we recommend a twofold strategy for mangrove protection and management. First, large forest patches should be vigorously protected because large habitats have the capacity to support rich
biodiversity and large populations, generally show higher resilience against environmental disturbances and have better prospects for species persistence in the long term (Ferraz et al., 2003; Ribeiro et al., 2009). This is corroborated by observations that F. cancrivora is most readily found in larger (>100 hm²) mangrove forests (Hong et al., 2011; our observation). However, maintaining large but spatially distant habitat fragments may create genetic isolation among populations, thus hampering the associated species’ metapopulation processes and evolutionary potential (Garant et al., 2007; Thomas, 2000). The strong genetic differentiation between population clusters in this study provides an example of such isolation. Therefore, our second suggestion is to preserve and restore a number of smaller (10–100 hm²) and geographically proximate mangrove patches along the coastlines. Smaller patches constitute a considerable fraction of remnant mangroves, often neglected in current conservation policy. Yet, they can play an important role in improving population viability in fragmented landscapes, either by functioning as movement corridors and stepping stones to facilitate gene flow between larger patches (Tewksbury et al., 2002; Uezu et al., 2008) or by forming networks of functionally connected habitat fragments, which would allow species such as F. cancrivora to persist in disturbed landscapes (Martensen et al., 2008; Ribeiro et al., 2009).

5 | CONCLUSION

Our results support that the F. cancrivora populations on the China coasts and in northern Vietnam are derived from the mangrove-type clade within the species complex. Population genetic analyses suggest that the species’ contemporary genetic patterns may have been shaped by both past and present coastal habitat configurations, and that current mangrove loss and fragmentation may have strong impacts on gene flow among habitat patches. Contrary to intuitive thought, F. cancrivora may have dispersed along the coast from Southeast Asia to China during Pleistocene glaciations when sea levels were low, colonizing the Hainan area first. This population subsequently spread to mainland China coasts when sea levels rose and shorelines withdrew. This study has important implications for understanding the population history of mangrove-associated biota and for the conservation management of this critical ecosystem.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Mitochondrial DNA sequences are available from GenBank (accession nos. MK396081–MK396095). Data used in this study are available from the authors upon request.

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REFERENCES

Alongi, D. M. (2002). Present state and future of the world’s mangrove forests. Environmental Conservation, 29, 331–349. https://doi.org/10.1017/s0376892902000231

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16, 37–48. https://doi.org/10.1093/oxfordjournals.molev.a026036

Beerli, P. (2006). Comparison of Bayesian and maximum likelihood inference of population genetic parameters. Bioinformatics, 22, 341–345. https://doi.org/10.1093/bioinformatics/bti803

Beerli, P., & Palczewski, M. (2010). Unified framework to evaluate panmixia and migration direction among multiple sampling locations. Genetics, 185, 313–326. https://doi.org/10.1534/genetics.109.112532

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., ... Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Computational Biology, 15, e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Cabrera, A. A., & Palsboll, P. J. (2017). Inferring past demographic changes from contemporary genetic data: A simulation-based evaluation of the ABC methods implemented in DIYABC. Molecular Ecology Resources, 17, e94–e110. https://doi.org/10.1111/1755-0998.12696

Chen, L., Wang, W., Zhang, Y., & Lin, G. (2009). Recent progresses in mangrove conservation, restoration and research in China. Journal of Plant Ecology, 2, 45–54. https://doi.org/10.1093/jpe/rtp009

Christy, M. T., Savidge, J. A., & Rodda, G. H. (2007). Multiple pathways for invasion of anurans on a Pacific island. Diversity and Distributions, 13, 598–607. https://doi.org/10.1111/j.1472-4442.2007.00378.x

Cole, T. L., Hammer, M. P., Unmack, P. J., Teske, P. R., Brauer, C. J., Adams, M., & Beheregaray, L. B. (2016). Range-wide fragmentation in a threatened fish associated with post-European settlement modification in the Murray-Darling Basin, Australia. Conservation Genetics, 17, 1377–1391. https://doi.org/10.1007/s10592-016-0868-8

Coleman, R. A., Gauffre, B., Pavlova, A., Beheregaray, L. B., Kearns, J., Lyon, J., Sasaki, M., Leblois, R., Sgro, C., & Sunnucks, P. (2018). Artificial barriers prevent genetic recovery of small isolated populations of a low-mobility freshwater fish, Hereditas, 120, 515–532. https://doi.org/10.1038/s41437-017-0008-3

Cornuet, J. M., & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144, 2001–2014. https://doi.org/10.1093/genetics/144.4.2001

Cornuet, J.-M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.-M., & Estoup, A. (2014). DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism. DNA sequence and microsatellite data. Bioinformatics, 30, 1187–1189. https://doi.org/10.1093/bioinformatics/btt763

Cornuet, J.-M., Ragnvé, V., & Estoup, A. (2010). Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC Bioinformatics, 11, 401.

Cornuet, J.-M., Santos, F., Beaumont, M. A., Robert, C. P., Marin, J.-M., Balding, D. J., Guillemaud, T., & Estoup, A. (2008). Inference
the rice frog (Fejervarya limnocharis) species complex from Sri Lanka, Thailand, Taiwan and Japan, inferred from mtDNA gene sequences, allozymes, and crossing experiments. Zoological Science, 24, 547–562. https://doi.org/10.2108/zsj.24.547
Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123, 585–595. https://doi.org/10.1093/genetics/123.3.585
Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30, 2725–2729. https://doi.org/10.1093/molbev/mst197
Tewksbury, J. J., Levey, D. J., Haddad, N. M., Sargent, S., Orrock, J. L., Weldon, A., Danielson, B. J., Brinkerhoff, J., Damschen, E. I., & Townsend, P. (2002). Corridors affect plants, animals, and their interactions in fragmented landscapes. Proceedings of the National Academy of Sciences of the United States of America, 99, 12923–12926. https://doi.org/10.1073/pnas.20224699
Thomas, C. D. (2000). Dispersal and extinction in fragmented landscapes. Proceedings of the Royal Society B-Biological Sciences, 267, 139–145. https://doi.org/10.1098/rspb.2000.0978
Toda, M., Matsu, M., Nishida, M., & Ota, H. (1998). Genetic divergence among Southeast and East Asian populations of Rana limnocharis (Amphibia: Anura), with special reference to sympatric cryptic species in Java. Zoological Science, 15, 607–613. https://doi.org/10.2108/zsj.15.607
Uchiyama, M., Murakami, T., & Yoshizawa, H. (1990). Notes on the development of the crab-eating frog, Rana cancivora. Zoological Science, 7, 73–78.
Uezu, A., Beyer, D. D., & Metzer, J. P. (2008). Can agroforest woodlots work as stepping stones for birds in the Atlantic forest region? Biodiversity and Conservation, 17, 1907–1922. https://doi.org/10.1007/s10531-008-9329-0
van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4, 535–538. https://doi.org/10.1046/j.1471-8286.2004.00684.x
Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. Journal of Biogeography, 27, 1153–1167. https://doi.org/10.1046/j.1365-2699.2000.00489.x
Wang, W., Qiao, Y., Li, S., Pan, W., & Yao, M. (2017). Low genetic diversity and strong population structure shaped by anthropogenic habitat fragmentation in a critically endangered primate, Trachypithecus leucocephalus. Heredity, 118, 542–553. https://doi.org/10.1038/hdy.2017.2
Wang, W. Q., & Wang, M. (2007). The Mangroves of China. Science Press.
Wright, S. (1943). Isolation by distance. Genetics, 28, 114–138. https://doi.org/10.1093/genetics/28.2.114
Yan, F., Zhou, W., Zhao, H., Yuan, Z., Wang, Y., Jiang, K., Jin, J., Murphy, R. W., Che, J., & Zhang, Y. (2013). Geological events play a larger role than Pleistocene climatic fluctuations in driving the genetic structure of Quasipaa boulengeri (Anura: Dicroglossidae). Molecular Ecology, 22, 1120–1133. https://doi.org/10.1111/mec.12153
Yao, Y., Harff, J., Meyer, M., & Zhan, W. (2009). Reconstruction of paleo-coastlines for the northwestern South China Sea since the Last Glacial Maximum. Science in China Series D-Earth Sciences, 52, 1127–1136. https://doi.org/10.1007/s11430-009-0098-8
Zhang, B., Edwards, O., Kang, L., & Fuller, S. (2014). A multi-genome analysis approach enables tracking of the invasion of a single Russian wheat aphid (Diuraphis noxia) clone throughout the New World. Molecular Ecology, 23, 1940–1951. https://doi.org/10.1111/mec.12714
Zhao, H., Wang, Y., Wang, Q., & Wang, M. (2016). Isolation and characterization of 20 microsatellite markers for the crab-eating frog Fejervarya cancivora. Conservation Genetics Resources, https://doi.org/10.1007/s12686-016-0546-7

BIOSKETCHES
The group mainly comprises molecular ecologists from China. Their research focuses on applying molecular tools to answer questions in population genetics, trophic interactions and biodiversity conservation. This work was part of a doctoral degree in Zoology by Y.Z. at Peking University.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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