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ORIGINAL ARTICLE

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Inferring the invasion history of coral berry *Ardisia crenata* from China to the USA using molecular markers

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Abstract Genetic comparisons between native and invasive populations of a species can provide insights into its invasion history information, which is useful for guiding management and control strategies. The coral berry *Ardisia crenata* was introduced to Florida last century as a cultivated ornament plant, and has since spread widely throughout the southern regions of the USA. Previously, the genetic variation among 20 natural populations of *A. crenata* across its distribution center in southern China was quantified using seven microsatellite markers. Here we expand on that work by additionally sampling individuals from four other native populations in Taiwan and Japan, and from five invasive populations in the USA. We also examined the results from one chloroplast intergenic spacer region (trnF-trnL) in all 29 populations. Our aim is to identify the invasion source and subsequent history of the species' spread throughout the southern USA. We observed lower genetic diversity in the invasive populations based on both microsatellite and chloroplast markers. Our data show that the invasive populations can be clustered with native populations in southeastern China, inferring this region as the geographic origin of *A. crenata* cultivars invading the USA. We further classified invasive individuals into invasive I and invasive II clusters. Nantou in Taiwan and Xihu in mainland China are the most closely related populations to those, which identify the former as potential sources for host-specific control agents. Our results, combined with the known introduction records, suggest that *A. crenata* was first multiply introduced into Florida and then secondarily colonized Louisiana and Texas from Florida.

Keywords *Ardisia crenata* · Invasive source · Introductions · Microsatellite · Chloroplast DNA

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Introduction

After introduction to a novel environment, alien species generally undergo some genetic alternation or evolution (Sakai et al. 2001); for example, many experience a loss of genetic diversity due to a founder effect following colonization (DeWalt and Hamrick 2004; Meimberg et al. 2006; Okada et al. 2009), while others increase their genetic diversity via hybridization with congeneric natives or outbreeding among multiple introduced populations (Genton et al. 2005; Lavergne and Molofsky 2007; Marrs et al. 2008). As such, comparing the genetic structure of alien and native populations of an invasive species can provide insights into its movement history, potential source populations, and effective control of invasive populations. In particular, such comparisons, which trace the origin(s) of invasive populations, can narrow the area over which to search for candidate species potentially useful for biological control (Goolsby et al. 2006; Ward et al. 2008; Okada et al. 2009).

Native to China, Japan, Korea, and northern India, the coral berry *Ardisia crenata* (Myrsinaceae) is an insect-pollinated evergreen subshrub found in subtropical

forests (Cheon et al. 2000). Within its main distribution in southern China, *A. crenata* is considered a traditional medicine used to treat several human diseases and parasites, and is widely cultivated as an ornamental plant (Chen 1979). *A. crenata* was introduced to Florida, USA in the early 1900s (Dozier 1999; Kitajima et al. 2006) and was renamed 'coral' or 'Christmas' berry due to its attractive red drupe fruit and shade tolerance in gardens (Bailey 1922; Conover and Poole 1989; Kitajima et al. 2006). However, some individuals escaped from cultivation, actively invaded natural ecosystems, and uncontrollably expanded to the understory of mesic forests in north-central Florida, Louisiana, and Texas; the species was also introduced to Hawaii (Singhurst et al. 1997; Bray et al. 2003). Introduced *A. crenata* individuals now grow at higher densities and are more shade-tolerant in their new range than in native areas, leading to the suppression of local understory plant species and the formation of dense, mono-dominant patches that cause substantial ecological and economic damage (Bray et al. 2003). Despite this general understanding, the finer-scale aspects of its invasion history (e.g., geographic origins and introduction times) are unknown. While either China or Japan is thought to be the source of invasive individuals (Lee et al. 2003; Kitajima et al. 2006), this assertion has not been tested quantitatively.

Nuclear microsatellite markers with codominant transmission are presumably neutral and cover extensive parts of the genome; thus, their isolation can provide much information on gene flow (Powell et al. 1996; Mu et al. 2010). Likewise, maternally inherited chloroplast DNA (cpDNA) are more conserved than nuclear markers and can provide information on the patterns of range expansion, which depends on seed dispersal (McCauley et al. 2003). Applying these two molecular markers to both invasive and native conspecifics can reveal elements of likely introduction history (Williams et al. 2005). Mu et al. (2010) examined the genetic variation of 20 natural populations of *A. crenata* across its distribution center in mainland of China using nuclear microsatellites. They determined from their sample that there is limited gene flow between populations and a high incidence of inbreeding, with strong population structure. Their results indicated that *A. crenata* in China can be divided into an eastern group and a western group according to genotype.

Here we add to this database by sampling native *A. crenata* individuals from other regions like Taiwan and Japan, and compare their genetic signatures to invasive individuals collected from the USA. We used the same microsatellite markers as did Mu et al. (2010) and further complement the genetic signature description based on cpDNA for all populations. Combined with the results from Mu et al. (2010), we aimed to: (1) compare the genetic diversity and population genetic structure between native and introduced wild populations of *A. crenata*, (2) identify the most likely origin(s) of the invasive populations, (3) test whether the invasive range resulted from single or multiple introductions, and finally (4) reconstruct the putative introduction and colonization routes of *A. crenata*.

Materials and methods

Sampling

We collected fresh leaves from 218 wild *A. crenata* individuals from four native populations (two in Taiwan and two in Japan) and five invasive populations in the USA (Fig. 1; Table 1) and dried them on silica gel. We extracted DNA using the modified CTAB method and stored it at −20 °C. We were unable to collect more than 13 individuals from two provinces (JAO and JAM) in Japan.

Chloroplast sequence analysis

We screened several individuals from different geographical regions initially with four cpDNA primer pairs: trnH-trnK, trnF-trnL, trnS-trnG, and trnD-trnH. Except for the trnF-trnL intergenic spacer region, the other regions were not polymorphic. Thus, we only used the trnF-trnL region to amplify DNA from either one or two individuals per population, including those sampled by Mu et al. (2010). We used polymerase chain reaction in a 40-μl solution containing 10 mM Tris–HCl (pH 8.4), 200 mM $(NH_4)_2SO_4$, 6 mM MgC_{l2}, 0.8 mM dNTPs, 0.8 μM primer, 200 ng of genomic DNA, and 1 U Taq polymerase (TaKaRa) on Eppendorf Master Cycles. Amplification protocols included an initial denaturing at 94 °C for 5 min, followed by 35 cycles of 50 s at 94 °C, 30 s at 49 °C of annealing and 90 s at 72 °C, and a final extension step for 10 min at 72 °C. We sequenced PCR product using Applied Biosystems' capillary sequencers (ABI3730), proofed and aligned sequences with Clustal x (Thompson et al. 1997), and identified haplotypes with BioEdit (Hall 1999).

Microsatellite analysis

We genotyped all individuals at seven microsatellite loci: Ac07, Ac26, Ac27, Ac29, Ac49, Ac53, and Ac63 using methods described by Hong et al. (2008) and Mu et al. (2010). We calculated the observed number of alleles (N_A), the number of effective alleles (N_E), observed heterozygosity (H_O), unbiased expected heterozygosity (UH_E), and the inbreeding coefficient (F_{IS}) per population using GENEALEX 6.2 (Peakall and Smouse 2006). We tested for linkage disequilibrium between all pairs of loci in each population with FSTAT 2.9.3 (Goudet 2001), and Hardy–Weinberg equilibrium with GENEPOP 4 (Raymond and Rousset 1995) with 10,000 permutations. We used FREENA (Chapuis and Estoup 2007) to estimate null allele frequency for each population and locus. We then compared the global Weir's F_{ST}

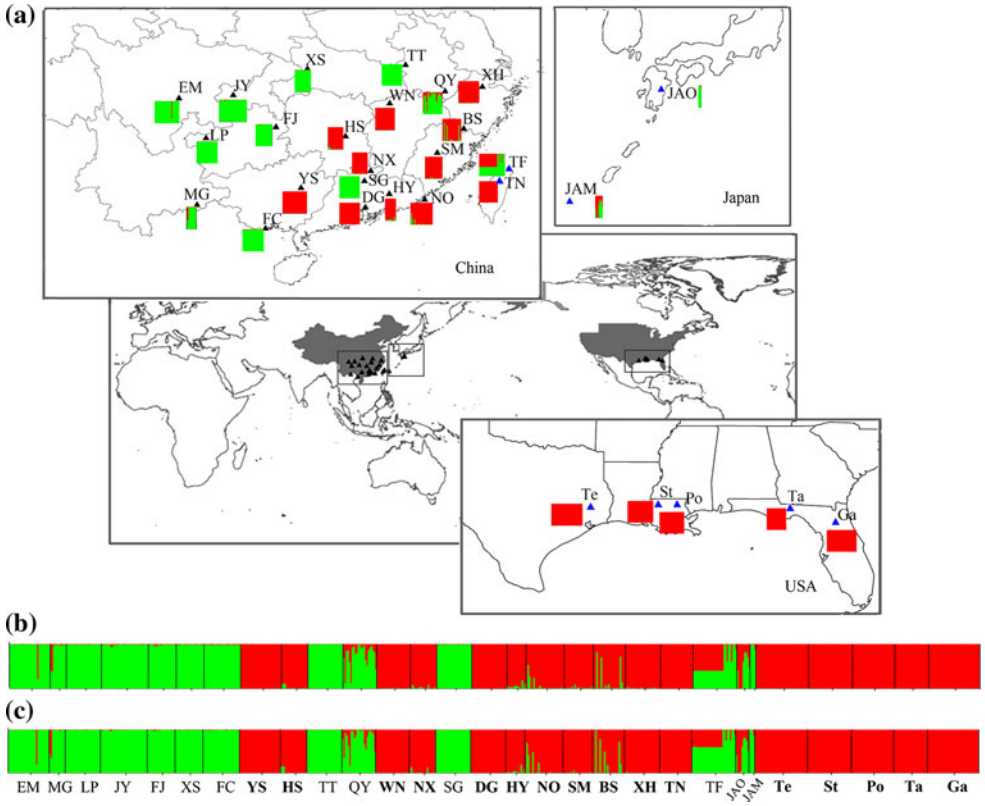

Fig. 1 Distribution map of sample sites and Bayesian clustering of all individuals in 29 populations. **a** Distribution of 29 populations and their assignment to two clusters from INSTRUCT. *Black triangles* indicate locations discussed in Mu et al. (2010) and *blue triangles* represent those additional sites we sampled. The *color bars* represent the assignment probability for each individual to a particular cluster (C1 and C2) using INSTRUCT (**b**) and STRUCUTRE (**c**). The populations marked in *bold* were selected for additional assignment tests (color figure online)

(Weir 1996) both with and without the excluding null alleles (ENA) correction (Chapuis and Estoup 2007) to determine the influence of null alleles.

We combined the microsatellite results from our nine sampled populations with the 20 populations sampled and described by Mu et al. (2010) to test whether native and invasive populations differed in their genetic signatures. We used FSTAT 2.9.3 (Goudet 2001) to calculate the number of private alleles at the population level and quantify statistically the difference in mean allelic richness (A_R, adjusted by the smallest sample size, $n = 13$), observed heterozygosity (H_O), gene diversity (H_s), and inbreeding coefficient (F_{IS}). JAM and JAO were not included in the analysis of genetic diversity among groups in FSTAT because their sample size was < 10. We calculated D_{est} coefficient (Jost 2008) between populations in SMOGD (V.1.2.5) software (Crawford 2010) to indicate their genetic differentiation. This estimation was more suitable to measure differentiation in those studies based on microsatellite markers (Heller and Siegismund 2009; Jost 2008, 2009). Mu et al. (2010) tested isolation by distance in mainland China populations with ARLEQUIN 3.11 (Excoffier et al. 2005), so we applied the same test to the invasive populations.

We implemented a Bayesian cluster analysis in STRUCTURE 2.3.1 (Pritchard et al. 2000; Falush et al. 2003) to assign individuals into clusters based on their multilocus genotypes. Using an admixture model with correlated allele frequencies among populations, we did ten independent runs for each K (putative cluster numbers, from 1 to 12) with 1,000,000 iterations after a burn-in period of 500,000 steps. Since $\ln\Pr(X|K)$ does not reliably identify the optimal number of clusters, we calculated another ad hoc criterion ΔK (Evanno et al. 2005) to determine the optimal K. We used CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) to calculate the average membership coefficient for each individual by aligning and converging the results of the above ten runs. Because all invasive individuals are assigned to one cluster identified by STRUTURE at the highest hierarchical level (Fig. 1), we did another analysis for the 16 populations which were completely assigned to this cluster (Fig. 1) in STRUCTURE with the same settings as above for K from 1 to 10 to understand the genetic structure and potential origin of the invasive populations in detail. In addition, we did a Bayesian cluster analysis using a more reasonable approach for inbreeding species, INSTRUCT (Gao et al. 2007), in

Table 1 Sites, sample size, and genetic variation of the 29 *Ardisia crenata* populations sampled

Code	Location	Longitude	Latitude	N	N_A	N_E	N_P	H_o	UH_E	F_{IS}
Native populations										
EM[a]	Emeishan, China	103°20′	29°33′	28	3.14	1.59	2	0.027	0.282	0.906**
MG[a]	Maguan, China	104°20′	23°06′	13	1.14	1.11	1	0.000	0.076	1.000**
LP[a]	Liupanshui, China	104°48′	27°27′	24	3.00	2.34	0	0.061	0.453	0.886**
JY[a]	Jingyunshan, China	106°22′	29°45′	32	6.00	4.06	2	0.233	0.741	0.682**
FJ[a]	Fanjingshan, China	108°45′	27°49′	19	3.43	2.37	2	0.150	0.424	0.652**
XS[a]	Xingshan, China	110°30′	31°21′	20	1.57	1.26	0	0.084	0.135	0.321**
FC[a]	Fangcheng, China	108°10′	21°40′	26	2.86	2.11	1	0.174	0.439	0.577**
YS[a]	Dayaoshan, China	110°09′	24°08′	28	1.43	1.18	0	0.036	0.098	0.639**
HS[a]	Henshan, China	112°38′	27°16′	18	1.43	1.19	1	0.034	0.097	0.660**
TT[a]	Tiantangzai, China	115°42′	31°12′	24	1.00	1.00	0	0.000	0.000	NA
QY[a]	Qiyunshan, China	118°02′	29°50′	23	4.43	2.99	4	0.440	0.573	0.243**
WN[a]	Wuning, China	115°06′	29°15′	23	2.57	1.94	2	0.119	0.397	0.714**
NX[a]	Nanqun, China	114°02′	25°10′	18	4.00	2.44	0	0.122	0.444	0.724**
SG[a]	Shaoguan, China	113°41′	24°33′	24	2.86	2.11	1	0.139	0.421	0.655**
DG[a]	Dongguan, China	113°44′	22°57′	24	1.29	1.11	3	0.048	0.082	0.413*
HY[a]	Heyuan, China	115°04′	23°46′	13	1.57	1.38	0	0.016	0.194	0.926**
NO[a]	Nanaodao, China	117°02′	23°25′	26	2.57	2.44	4	0.055	0.450	0.878**
SM[a]	Saming, China	117°47′	26°15′	20	2.29	1.40	1	0.132	0.234	0.448**
BS[a]	Baishanzu, China	119°14′	27°43′	22	4.00	2.04	6	0.120	0.455	0.740**
XH[a]	Xihu, China	120°16′	30°15′	24	2.00	1.57	0	0.048	0.245	0.809**
TN	Nantou, Taiwan, China	120°68′	23°92′	22	1.29	1.09	0	0.054	0.067	0.212
TF	Fushan, Taiwan, China	121°50′	24°78′	30	3.14	1.75	4	0.152	0.390	0.620**
JAO	Okinawa, Japan	123°75′	24°41′	9	3.14	2.17	0	0.095	0.465	0.816**
JAM	MiyaZaki, Japan	131°42′	31°93′	4	1.86	1.52	0	0.179	0.246	0.400**
Invasive populations										
Te	Texas, USA	−94°08′	30°06′	36	1.14	1.01	0	0.000	0.008	1.000*
St	St. Francisville, USA	−91°35′	30°78′	30	1.00	1.00	0	0.000	0.000	NA
Po	Ponchatoula, USA	−90°34′	30°44′	29	1.71	1.21	0	0.020	0.163	0.883**
Ta	Tallahassee, USA	−84°34′	30°46′	23	1.71	1.30	0	0.068	0.199	0.668**
Ga	Gainesville, USA	−82°34′	29°66′	35	1.71	1.17	0	0.008	0.125	0.936**

N sample size, N_A observed alleles number, N_E number of effective alleles, N_P number of private alleles, H_O observed heterozygosity, UH_E unbiased expected heterozygosity, F_{IS} inbreeding coefficient

** Denote deviation from Hardy–Weinberg equilibrium after Bonferroni correction

[a]The populations quoted in Mu et al. (2010)

which individuals can be assigned to clusters by calculating expected genotype frequencies on the basis of inbreeding or selfing rates without the assumption of Hardy–Weinberg equilibrium. We did five independent runs on mode 4 (inferring population structure and population inbreeding coefficients) in INSTRUCT, and other parameters in INSTRUCT were identical to those described in STRUCTURE. To identify the optimal model of K in INSTRUCT, we also provided the deviance information criterion (DIC).

We also applied another Bayesian assignment test of each invasive individual to each native population developed by Rannala and Mountain (1997) and implemented in GENECLASS2 (Piry et al. 2004) to discover the most likely origin(s) of the invasive individuals. This method gives a score (the percentage of likelihood value of an individual assigned to a reference population in the sum of likelihood values of all individuals to that population) and ranks them in decreasing order. Thus, we considered high-score assignments (>0.99) as a credible result, but those with scores <0.99 as an uncertain assignment. We determined the assignment score for each invasive individual clustered to each native population via 10,000 Markov chain-Monte Carlo simulations in GENECLASS2.

Results

Chloroplast haplotypes

We identified three haplotypes (840 bp) in the native and invasive populations for the trnF-trnL intergenic spacer. The most common haplotype H1 was fixed in all exotic USA populations and in all but two of the native populations (MG and LP) in southwest China. Haplotype H2 with a transition substitution (C–T) was unique in the LP population, and H3 with four transition substitutions (T–C, A–G, T–C and T–C) and four inserts (ATA, G, AT and TTT) was fixed in the MG population.

Genetic diversity and structure

From the four additional native and five invasive populations we sampled, we identified a total of 49 alleles in

the seven microsatellite loci. We observed heterozygote deficits ($H_O < UH_E$) in each population except St, and F_{IS} (0.212–1.000) was high. As for the other 20 populations in mainland China analyzed by Mu et al. (2010), all the populations we sampled (except TN) deviated from Hardy–Weinberg equilibrium (Table 1). There was no consistent pattern of linkage disequilibrium at locus pairs. About 33.5 % of the null allele frequencies were >0.2 for each population and locus. The global measure of Weir's F_{ST} changed slightly from 0.637 to 0.621 after ENA correction.

After combining our sampled populations with the other 20 from mainland China analyzed by Mu et al. (2010), we found evidence that mean allelic richness (A_R), observed heterozygosity (H_O), and gene diversity (H_s) were lower in the invasive compared to native populations (Table 2), while the mean inbreeding coefficients (F_{IS}) were not (Table 2). In the native regions, we detected 17 private alleles in the populations with their sample size no more than 23 (the smallest invasive population size), but none in the invasive populations (Table 1).

Most of the total genetic variation (>50 %) was among populations, regardless of whether we treated populations as nested within regions or separately in native and invasive regions, and 16.3 % of it was attributable to region (Table 3). D_{est} for population pairs ranged from 0.001 to 1, with the three smallest D_{est} between pairs of three invasive populations St, Po, and Ga (Table 4). A Mantel test indicated there was no evidence for a relationship between genetic distance and spatial distance in the USA ($r = 0.049$, $p = 0.400$).

Table 2 Statistical comparison of genetic diversity between native and invasive *A. crenata*

	N	A_R	H_O	H_s	F_{IS}
Native (G1)	508	1.309	0.112	0.324	0.653
Invasive (G2)	153	1.101	0.016	0.094	0.831
One-tailed type I error probabilities (G1 > G2)		0.010	0.004	0.009	0.920

A_R allelic richness, H_O observed heterozygosity, H_S gene diversity, F_{IS} inbreeding coefficient

Assignment analysis

The assignment results from INSTRUCT and STRUCTURE are highly congruent. With *K* increasing from 1 to 12 in the assignment of all individuals, the deviance information criterion in INSTRUCT decreased and $\ln\Pr(X|K)$ in the STRUCTURE algorithm increased monotonically, and both of their ΔK were highest at $K = 2$ (Table 5). Thus, we conclude that there is most support for two genetic groups: individuals from western China were assigned to the green cluster (C1), and invasive individuals from the USA, native individuals from southeastern China, and part of individuals from Japan formed another red cluster (C2) (Fig. 1). Additional assignment for 16 populations of C2 indicated that two clusters still best categorized the individuals within this subset (Table 5). Here, invasive individuals were assigned to two clusters. All individuals from St and most from Po and Ga belonged to the pink 'Invasive I' cluster (Fig. 2); the remaining invasive individuals including all of Te and most of Ta were assigned to the yellow 'Invasive II' cluster (Fig. 2). The Invasive I cluster grouped best with the native populations DG and TN, while the Invasive II cluster grouped best with XH, SM, etc. (Fig. 2).

The detection of migration ancestry showed that invasive individuals were mainly assigned to two native populations with the highest score >0.99: most of the invasive I clustered to TN, and most of the invasive II clustered to XH (Fig. 3). The remaining assignments

Table 3 Summary of the analysis of molecular variance (AMOVA) for *A. crenata* populations

Source of variation	*df*	Sum of squares	Variance components	Variation (%)
Native and Invasive regions				
Among regions	1	211.538	0.321	16.32
Among populations within regions	26	1,249.635	1.004	51.01
Among individual within populations	635	675.225	0.420	21.33
Within individuals	663	148.000	0.223	11.34
Total	1,325	2,284.397	1.969	
Native regions				
Among populations	22	655.821	0.655	56.70
Among individuals within populations	487	395.900	0.312	27.02
Within individuals	510	96.000	0.188	16.28
Total	1,019	1,147.722	1.156	
Invasive regions				
Among populations	4	147.028	0.599	75.38
Among individuals within populations	148	50.613	0.146	18.45
Within individuals	153	7.500	0.049	6.17
Total	305	205.141	0.794	

All variance components were non-random ($p < 0.0001$) as determined from permutation tests

Table 4 D_{est} between 29 sample sites from native and invasive regions

	EM	MG	LP	JY	FJ	XS	FC	YS	HS	TT	QY	WN	NX	SG	DG	HY	NO	SM	BS	XH	TN	TF	JAO	JAM	Te	St	Po	Ta	Ga
EM	–																												
MG	0.44	–																											
LP	0.52	0.82	–																										
JY	0.49	0.52	0.26	–																									
FJ	0.81	0.73	0.41	0.38	–																								
XS	0.80	0.50	0.43	0.56	0.13	–																							
FC	0.65	0.49	0.80	0.57	0.61	0.79	–																						
YS	0.72	0.72	1.00	0.74	0.79	0.92	0.54	–																					
HS	0.71	0.50	0.96	0.70	0.85	0.69	0.71	0.30	–																				
TT	0.87	0.81	0.46	0.54	0.31	0.49	0.89	1.00	0.99	–																			
QY	0.66	0.45	0.82	0.67	0.88	0.94	0.58	0.62	0.54	1.00	–																		
WN	0.71	0.65	0.97	0.65	0.66	0.77	0.52	0.32	0.26	0.69	0.47	–																	
NX	0.71	0.43	0.76	0.62	0.77	0.87	0.66	0.40	0.22	0.88	0.56	0.43	–																
SG	0.69	0.35	0.79	0.72	0.84	0.63	0.19	0.66	0.66	1.00	0.53	0.69	0.64	–															
DG	0.72	0.72	1.00	0.75	0.87	0.96	0.72	0.31	0.49	1.00	0.69	0.49	0.62	0.72	–														
HY	0.59	0.67	0.74	0.63	0.67	1.00	0.61	0.38	0.38	0.58	0.53	0.13	0.30	0.72	0.49	–													
NO	0.69	0.54	1.00	0.57	0.62	0.82	0.63	0.34	0.22	0.61	0.53	0.16	0.32	0.66	0.42	0.17	–												
SM	0.65	0.65	0.77	0.56	0.57	0.96	0.46	0.23	0.39	0.76	0.59	0.26	0.25	0.66	0.59	0.18	0.29	–											
BS	0.69	0.59	0.77	0.77	0.93	0.92	0.68	0.51	0.49	0.92	0.37	0.31	0.27	0.65	0.39	0.25	0.35	0.38	–										
XH	0.71	0.58	0.80	0.59	0.63	0.87	0.44	0.22	0.22	0.79	0.59	0.26	0.24	0.59	0.39	0.14	0.23	0.10	0.26	–									
TN	0.72	0.67	0.83	0.81	0.94	1.00	0.72	0.48	0.35	1.00	0.47	0.37	0.31	0.72	0.30	0.22	0.39	0.36	0.17	0.15	–								
TF	0.78	0.82	0.94	0.63	0.49	0.88	0.53	0.59	0.81	0.72	0.73	0.57	0.75	0.73	0.83	0.56	0.57	0.41	0.82	0.50	0.82	–							
JAO	0.78	0.73	0.77	0.61	0.57	0.97	0.56	0.31	0.34	0.72	0.48	0.24	0.34	0.73	0.53	0.17	0.21	0.19	0.39	0.16	0.39	0.42	–						
JAM	0.51	0.75	0.89	0.63	0.72	1.00	0.65	0.76	0.75	0.65	0.56	0.54	0.69	0.75	0.78	0.49	0.52	0.55	0.75	0.58	0.78	0.51	0.14	–					
Te	0.72	0.67	0.84	0.63	0.54	0.96	0.47	0.17	0.20	0.72	0.58	0.24	0.25	0.66	0.49	0.11	0.21	**0.05**	0.36	**0.04**	0.18	0.38	0.13	0.52	–				
St	0.72	0.72	1.00	0.77	0.96	0.96	0.72	0.30	0.49	1.00	0.52	0.32	0.48	0.72	0.17	0.38	0.38	0.40	0.29	0.39	**0.06**	0.82	0.51	0.78	0.48	–			
Po	0.72	0.71	0.98	0.74	0.90	0.95	0.69	0.29	0.46	0.97	0.50	0.28	0.42	0.71	0.17	0.32	0.34	0.35	0.25	0.32	**0.05**	0.78	0.46	0.75	0.41	**0.008**	–		
Ta	0.72	0.71	0.99	0.57	0.53	0.92	0.45	0.08	0.25	0.72	0.59	0.17	0.33	0.66	0.30	0.19	0.18	**0.07**	0.45	0.10	0.31	0.38	0.15	0.52	0.05	0.18	0.14	–	
Ga	0.72	0.71	0.99	0.76	0.92	0.95	0.69	0.27	0.46	1.00	0.50	0.27	0.45	0.71	0.18	0.33	0.33	0.35	0.24	0.32	**0.07**	0.79	0.45	0.77	0.42	**0.004**	**0.001**	0.15	–

D_{est} <0.1 is marked in *bold*

Table 5 Criteria from the Bayesian cluster analysis for selecting the optimal *K*

	Assignment of all individuals					Addition assignment of individuals in C2				
	INSTRUCT			STRUCTURE		INSTRUCT			STRUCTURE	
K	DIC	lnPr(*X*|*K*)	Δ*K*	lnPr(*X*|*K*)	Δ*K*	DIC	lnPr(*X*|*K*)	Δ*K*	lnPr(*X*|*K*)	Δ*K*
1	20783.9	−10392.0	–	−16737.6	–	7523.2	−3761.6	–	−6169.1	–
2	16666.4	−8327.3	**45.29**	−13150.8	**45.75**	5818.5	−2910.0	**216.84**	−4711.7	**122.38**
3	15036.4	−7565.7	0.89	−11853.3	0.76	4985.8	−2492.9	5.15	−4021.7	3.81
4	13775.7	−6891.6	1.51	−10662.3	4.35	4511.3	−2255.7	1.96	−3595.9	1.80
5	12750.9	−6361.3	2.35	−9992.4	0.60	4122.8	−2055.3	2.26	−3250.8	0.16
6	11924.7	−5962.8	3.43	−9236.9	2.41	3778.2	−1889.1	1.82	−2921.3	1.75
7	11490.5	−5739.2	0.59	−8741.5	0.89	3528.9	−1779.5	0.81	−2722.7	2.23
8	10908.4	−5472.8	0.94	−8387.4	0.77	3410.5	−1683.2	4.13	−2674.2	8.70
9	10495.7	−5271.8	0.16	−7867.2	0.63	3281.4	−1651.1	0.93	−2494.0	6.13
10	10120.5	−5062.4	0.87	−7438.1	2.84	3196.6	−1591.7	–	−2300.2	–
11	9825.4	−4921.2	0.27	−7167.8	0.15					
12	9586.4	−4764.3	–	−6876.1	–					

The top-ranked *K* model within each class is marked in *bold*

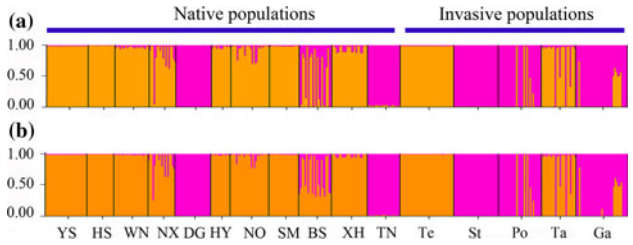

Fig. 2 Additional assignments of individuals from 16 populations in C2 assessed by **a** INSTRUCT and **b** STRUCTURE. Each *color* represents a different genetic cluster (color figure online)

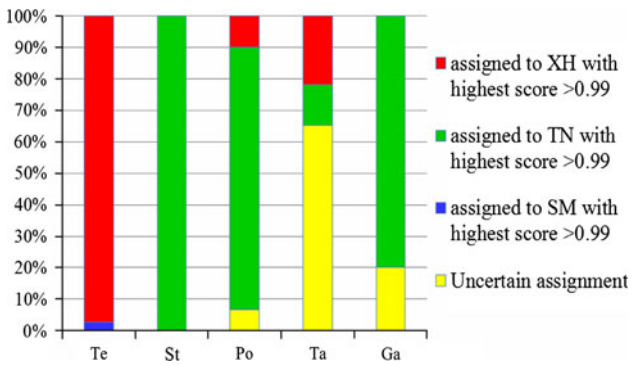

Fig. 3 Percentage of individuals of each invasive population clustered to each of the most likely invasive origins by GENECLASS2. 'Uncertain' indicates assignments without a credible result

were with low assignment significance, which we called 'uncertain' assignment (Fig. 3), but the highest scores for most of them were still assigned to XH, TN, or SM, and only three individuals from Ga were uncertainly assigned to NX with the highest score < 0.50.

Discussion

Using chloroplast DNA markers, we essentially eliminated two native populations (MG and LP) in southwest China as the likely source of the invasive coral berry introduction to the USA. Microsatellite analysis confirmed that conclusion, and further indicated that the invasive populations most likely originated from southeast China rather than from Japan or southwest China. The problem of invasive species is typically linked to the growth and development of trade, transport, and travel (Dehnen-Schmutz et al. 2007). Southeast China always has an active economy, especially within the last few decades, with frequent trade along its coasts. As a commercial ornament plant, *A. crenata* dispersal pathways should arise mainly from the pattern of predominant trade routes. Lee et al. (2003) found that *A. crenata* individuals in the USA had more similar origins to those marketed in Korea, rather than to native populations in Korea or Japan. Therefore, long-distance and cross-continental dispersal of *A. crenata* most likely occurred from China via the Korean market to the USA, and perhaps also via the Japanese market because of their relatively short geographic distance and the high frequency trade between Japan and Korea, although that hypothesis requires more data to test definitively.

Cultivated populations usually have a complex demographic and genetic history due to artificial hybridization and multiple sites and timing of introduction—this is common among ornamental plants such as *Hypericum perforatum, Pueria lobata*, and *Schinus terebinthifolius* (Maron et al. 2004; Sun et al. 2005; Williams et al. 2005). Roh et al. (2006) did find that a cultivar of *A. crenata* was hybridized from other cultivars. Indeed, we observed two genetic clusters for the invaded individuals, indicating there were at least two genetic sources in the invasive region.

As expected from founding populations of a few individuals (Novak and Mack 2005), we found that genetic diversity was lower in individuals sampled from the invasive region compared to native individuals based on both chloroplast DNA and microsatellite markers. We also observed heterozygote deficits and strong genetic structure in both native and invasive populations. Null allele is a common cause of heterozygote deficit in microsatellites (Selkoe and Toonen 2006). However, we found that the global F_{ST} changes negligibly after correcting for the occurrence of null alleles. Mu et al. (2010) also discussed this genetic pattern in the native region of southern China and attributed heterozygote deficits to the species' self-compatible mating system leading to high incidences of inbreeding within populations. The high probability of inbreeding in the species is likely due to the connivent stamens that facilitate intrafloral self-pollination (Pascarella 1997) and the low densities at which native populations are observed (Mu et al. 2010). Inbreeding can lead to heterozygote deficits and high genetic variation among populations (Hamrick and Godt 1996; Nybom 2004). In our study, we therefore conclude that inbreeding, and not the occurrence of null alleles, contributes the most to genetic diversity and structure in *A. crenata*.

We also found stronger genetic structure after the invasion occurred. Over 75 % of the variation occurred between sampling locations in the invasive range, but just 56 % in native ranges. Moreover, the patterns of isolation by distance disappeared in the invasive populations, unlike in native regions (Mu et al. 2010). Natural dispersal of *A. crenata* is mainly provided by bird foragers of the drupe fruits, but Kitajima et al. (2006) found that avian dispersal is less effective in the invaded compared to the native range. Therefore, our observation of increased genetic differentiation in invaded regions is reasonable considering the more limited natural dispersal and consequential lower gene flow. Because of its ornamental characteristics, it is possible that human transport leads to long-distance dispersal, regardless of geographic distance. The pattern of low natural dispersal coupled with human-mediated long-distance spread has been reported in other invasive species such the tunicate *Botrylloides violaceus* (Bock et al. 2011).

We were careful to sample all native individuals from natural areas, but it is still possible that some native individuals we sampled were in fact the progeny of cultivar outbreaks. If this sort of sourcing error occurred, we would expect the clustering results to provide false, or at least weakened, conclusions regarding the source of the invasive individuals. Regardless, there is no evidence of an uncontrolled expansion of cultivars of *A. crenata* in their native region; therefore, our conclusion that the native southeastern Chinese populations XH and TN were the most likely origin populations for the two clusters identified in the invasive region still provides useful information for the identification of host-specific control agents or the search for the key differences for invasion success.

Historical records indicate that *A. crenata* was first imported into Florida as an ornamental in the early 1900s (Dozier 1999; Kitajima et al. 2006). It was first observed in natural moist areas in northern Florida in 1982 (Wunderlin 1982), and subsequently in other areas of the southern USA (e.g., Texas) in 1997 (Singhurst et al. 1997; Langeland and Burks 1998). Thus, it is likely that Florida was the initial site of invasion and spread. Initial colonizers typically have higher genetic diversity than secondary colonist populations (Rosenthal et al. 2008), an expectation that aligns well with our results (Table 1). The assignment result both from INSTRUCT and GENECLASS2 tests indicate that there is probably a more complex genetic component in Florida than in other invasive regions, supporting the assumption of multiple introductions in Florida. A close genetic relationship between Ga, Po, and St suggests that the Gainesville population (Florida) is the most likely source of the populations Po and St in Louisiana. However, the Texas population clusters to Ta in Florida instead of its nearby populations in Louisiana, suggesting secondary colonization from Florida to Texas. This could arise via a human-mediated dispersal that led to direct introduction from Florida to Texas.

It is notable that humans are the mechanism for long-distance dispersal of *A. crenata* for both before and after invasion. Constraining the transmission of such species by human endeavor therefore is likely the most tractable means to restrict introduction and establishment success. Genetically, homogeneous populations are usually vulnerable to biological control agents (Van Driesche and Bellows 1996); therefore, identifying host-specific control agents for *A. crenata* from the most likely origin could represent an effective mitigating intervention, especially for controlling populations in Texas and Louisiana. A combination of biological and physical/chemical control is likely to be the most effective interventions for the Florida populations.

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