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Population genetic structure of three tree species in the mangrove genus *Ceriops* (Rhizophoraceae) from the Indo West Pacific

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Abstract *Ceriops* is a viviparous mangrove with widespread species *Ceriops decandra* and *C. tagal*, and an endemic species *C. australis*. Genetic diversity of the three species was screened in 30 populations collected from 23 locations in the Indo West Pacific (IWP) using Inter-simple sequence repeats (ISSR) and sequences of partial nuclear gene (*G3pdh*) and chloroplast DNA (*trnV-trnM*). At the species level, the total gene diversity (Ht) revealed by ISSRs was 0.270, 0.118, and 0.089 in *C. decandra*, *C. tagal*, and *C. australis*, respectively. A total of six haplotypes of *G3pdh* and five haplotypes of *trnV-trnM* were recognized among the three species. Only *C. decandra* was detected containing more than one haplotype from each sequence data set (four *G3pdh* haplotypes and three *trnV-trnM* haplotypes). At the population level, genetic diversity of *Ceriops* was relatively low inferred from ISSRs (He = 0.028, 0.023, and 0.053 in *C. decandra*, *C. tagal*, and *C. australis*, respectively). No haplotype diversity within population was detected from any of the three species. Cluster analysis based on ISSRs identified three major geographical groups in correspond to the East Indian Ocean (EIO), South China Sea (SCS), and North Australia (NA) in both *C. decandra* and *C. tagal*. The cladogram from DNA sequences also detected the same three geographical groups in *C. decandra*. Analysis of molecular variance (AMOVA) revealed that most of the total variation was accounted for by differentiation between the three major geographical regions of both *C. decandra* and *C. tagal*. The significant genetic structure may result from the geological events in these regions during the recent Pleistocene glaciations. This study also provided insights into the phylogenetics of *Ceriops*.

Keywords *Ceriops australis* · *C. decandra* · *C. tagal* · Genetic structure · *G3pdh* · ISSR · *trnV-trnM*

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Introduction

Mangrove forests are the most dominant intertidal ecosystems along the tropical and subtropical coastlines. *Ceriops* Arnold (Rhizophoraceae), the "Yellow Mangrove," is one of the typical constituents of the inner mangrove, characterized by vivipary (i.e., the development of the progeny when still attached to the maternal parent) (Tomlinson 1986). This genus comprises three species, *Ceriops decandra*, *C. tagal*, and *C. australis* (Ballment et al. 1988; Duke 1992). Indeed, *C. australis* was previously treated as a variety of *C. tagal*, *C. tagal* var. *australis*, but more recently, a reclassification of *C. tagal* var. *australis* as *C. australis* was reported based on allozyme analysis (Ballment et al. 1988). Geographically, *C. australis* has a rather limited distribution area endemic in the littoral zone of Australasia (i.e., Australia, New Zealand, and New Guinea). *C. tagal* is widespread in the most extensive range throughout the tropics of the Indo West Pacific (IWP) region, whereas

C. decandra is native to a narrower range in this region (including Australasia and Indo-Malesia, but not extensive to East Africa) (Duke 1992).

The IWP is one of the major biogeographic global regions of mangrove distribution, which encompasses two potential discontinuities: the Malay Peninsula, and the Indonesia archipelago (including New Guinea), which lie on a shallow continental shelf that has been exposed repeatedly to create land connections in relatively recent geological history (Benzie 1998; Voris 2000). It was reported that past climatic and geomorphological events may have shaped population genetic structure through their effects on species distributions and levels of gene flow among populations (Benzie 1998; Dodd et al. 2002). Mangrove species whose propagules are disseminated in the oceans provide an excellent model for studying the effects of historical biogeographical events on population genetic structure. *C. decandra* and *C. tagal*, with their wide geographical distributions within the IWP region, are a good model affording the opportunity to investigate genetic differentiation at different spatial scales that integrate various historical biogeographical events.

Molecular markers, such as allozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites (SSR), Inter-simple sequence repeats (ISSR), and DNA sequence, have proven to be a very efficient means to investigate population genetics of mangrove species (Dodd et al. 2002; Maguire et al. 2000; Jian et al. 2004; Sun et al. 1998; Chiang et al. 2001; Tang et al. 2003; Takayama et al. 2006). A study on *C. tagal* using ISSR from Thailand and South China presented low-genetic variation at the population level (He = 0.008); however, a relatively high genetic variation (S. Huang, personal communication) within population was found using isozyme analyses (He = 0.195); more recently, cpDNA also revealed a high average haplotype diversity (Hd = 0.549) of total populations in *C. tagal* from Malay Peninsula and Borneo (Liao et al. 2006).

Here, a comparative study was conducted on the population genetic structure of the three species of *Ceriops* within the IWP region using different molecular markers. We selected DNA sequence fragments, the *trnV-trnM* and *G3pdh*, and ISSR as genetic markers. The *trnV-trnM* is a cpDNA intergenic spacer as described by Taberlet et al. (1991), whereas the *G3pdh* is a portion of a single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Strand et al. 1997). Both of the two fragments have been applied in some plant population genetic and phylogeographic studies (e.g., Huang et al. 2002; Hwang et al. 2000; Olsen and Schaal 1999; Olsen 2002). ISSR (Inter-simple sequence repeat) analysis from selective amplification of genomic DNA was also used in this study due to the high polymorphism usually found at ISSR loci in genetic diversity studies (Zietkiewicz et al. 1994; Gupta et al. 1994). We tried to address the following questions: (1) what is the genetic diversity and population structure in the three species of *Ceriops* and how efficient are ISSR and DNA sequence markers in studying these species? (2) Has the historical biogeographical events in the IWP had an important influence on population genetic structure of the widespread species *C. decandra* and *C. tagal*? and (3) what can be inferred from these data that has implications for the phylogenetics of this genus?

Materials and methods

Sample collection and DNA extraction

A total of 385 individuals of leaf samples across three *Ceriops* species were collected from 30 natural populations in the IWP, including three geographical regions, i.e., the East Indian Ocean (EIO), the South China Sea (SCS), and North Australia (NA) (Table 1; Fig. 1). The distance of sampled trees is at least 30 m. Total samples for ISSR analysis consisted of 189 individuals from 13 populations of *C. decandra*, 152 from 14 populations of *C. tagal*, and 44 from 3 populations of *C. australis*. The sample number for DNA sequencing was 107, 117, and 28 from *C. decandra*, *C. tagal*, and *C. australis*, respectively. Genomic DNA of each individual was extracted using the CTAB method (Doyle and Doyle 1987).

PCR amplification of *G3pdh* and *trnV-trnM*, and DNA sequencing

The *G3pdh* region was amplified with primers GPDX7F and GPDX9R following Olsen (2002). The same primers were used for sequencing. For *trnV-trnM* spacer, PCR was performed following Huang et al. (2002) with the forward primer *trnV* and the reverse primer *trnM*. In addition to the two external primers, two internal primers were used in sequencing: *trnV-2* (5′ TGGGCTCTTTCAATAACT 3′) and *trnM-2* (5′ TTGATTACATGATATACTCCT 3′), forward and reverse, respectively. PCR products were purified using agarose gel purification Kit (QIAGEN, Hilden, Germany) following the protocol provided by the manufacturers. Sequences were analyzed with the ABI PRISM 3700 sequencer.

ISSR amplification

One hundred ISSR primers from the Biotechnology Laboratory, at the University of British Columbia (UBC

Table 1 Population data

Region group	Location	*C. decandra*		*C. tagal*		*C. australis*	
		Pop. Code	He	Pop. Code	He	Pop. Code	He
SCS	Bangkok, Thailand	CD1[a] (20,10)[b]	0.014	–		–	–
SCS	Khanom, Thailand	CD2(19,10)	0.007	CT2(12,11)	0.020	–	–
SCS	Kukup, Malaysia	CD6(18,10)	0.038	CT6(12,10)	0.016	–	–
SCS	Tanjung Piai, Malaysia	CD7(7,7)	0.024	–		–	–
SCS	Pasir Ris, Singapore	CD8(16,10)	0.029	–		–	–
SCS	Tanmen, Hainnan, South China	–		CT14(10,8)	0.003	–	–
SCS	Yalongwan, Hainnan, South China	–		CT15(10,8)	0.004	–	–
SCS	Lingao, Hainnan, South China	–		CT16(10,8)	0.013	–	–
SCS	Dongge, Hainnan, South China	–		CT17(9,8)	0.004	–	–
SCS	Dongzhaigang, Hainnan, South China	–		CT18(10,8)	0.003	–	–
SCS	Kuching, Indonesia	CD22(6,4)	0.016	CT22(8,5)	0.019	–	–
SCS	Tuaran, Indonesia	CD23(11,6)	0.014	CT23(8,5)	0.021	–	–
EIO	Lu-un, Thailand	CD3(17,10)	0.037	–		–	–
EIO	Phang-Nya, Thailand	CD4(22,10)	0.033	CT4(15,9)	0.042	–	–
EIO	Ranong, Thailand	CD5(20,10)	0.059	CT5(12,9)	0.021	–	–
EIO	Godavari delta, India	CD19(9,4)	0.021	–		–	–
EIO	Pambala, Sri Lanka	–		CT21(10,8)	0.023	–	–
EIO	Neganbe, Sri Lanka	–		CT20 (10,8)	0.021		
NA	Daintree River (A), north Australia	CD9(12,8)	0.022	CT9(16,12)	0.037	–	–
NA	Daintree River (B), North Australia	CD10(12,8)	0.018	–		–	–
NA	Cairns airport, North Australia	–		–		CA11(10,8)	0.039
NA	Channel Island, North Australia	–		–		CA12(20,10)	0.029
NA	Channel Island Driveway, North Australia	–		–		CA13(14,10)	0.036
Mean			0.028		0.023		0.053
At the species level		13 populations (189, 107)[b]	0.270	14 populations (152, 117)	0.118	3 populations (44, 28)	0.089

Regional groups are determined from clustering analysis (see text, Fig. 2)

He the expected heterozygosity, *CD C. decandra*, *CT C. tagal*, *CA C. australis*. *SCS* South China Sea, *EIO* East Indian Ocean, *NA* North Australia

[a] The population code. For the location number see Fig. 1

[b] The sample number for ISSRs, and for sequencing, respectively

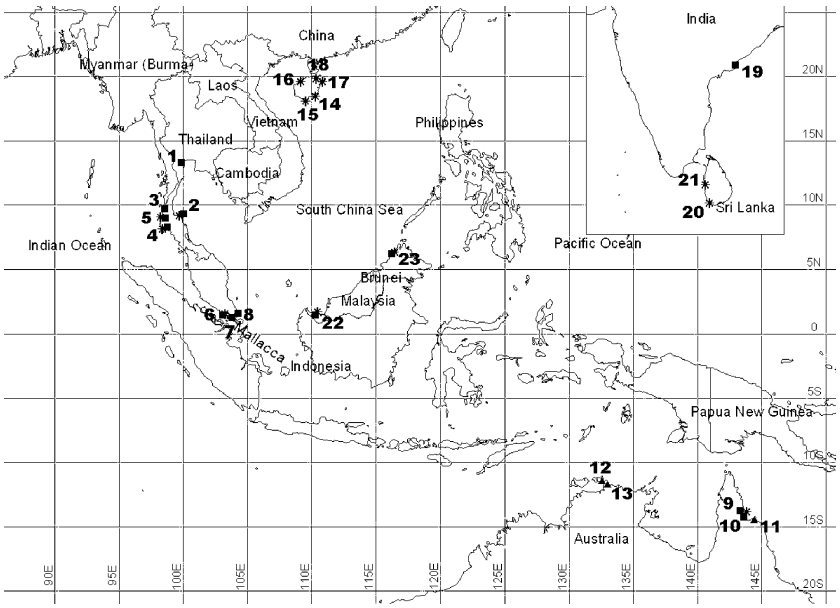

Fig. 1 Map showing locations of *Ceriops* populations sampled. *Filled squares* are sampled locations of *C. decandra*; *asterisks* are sampled locations of *C. tagal*; *filled triangles* are sampled locations of *C. australis.* Population names are 1. Bangkok; 2. Khanom; 3. Lu-un; 4. Phang-Nya; 5. Ranong; 6. Kukup; 7. Tanjung Piai; 8. Pasir Ris; 9. Daintree River (A); 10. Daintree River (B); 11. Cairns airport; 12. Channel Island; 13. Channel Island Driveway; 14. Tanmen; 15. Yalongwan; 16. Linggao; 17. Dongge; 18. Dongzhaigang; 19. Godavari delta; 20. Neganbe; 21. Pambala; 22. Kuching; 23. Tuaran

set no. 9) were initially screened and eleven of the primers that produced clear and reproducible fragments were used: 807 (AG)8T, 808 (AG)8C, 810 (GA)8T, 825 (AC)8T, 834 (AG)8YT, 835 (AG)8YC, 841 (GA)8YC, 842 (GA)8YG, 857 (AC)8YG, 889 DBD(AC)7, and 891 HVH(TG)7. PCR amplifications were performed in 10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl2, 0.1 mM dNTPs, 0.1% Triton X-100, 2% formamide, 200 nM primer, 1 U of Taq polymerase, and 20 ng of genomic DNA per 10 μL reaction. Amplification was performed in a PTC-200 thermocycler under the following cycle profile: initial denaturation at 94°C for 5 min, followed by 45 s at 94°C, 45 s annealing at 52–56°C, and 1.5 min extension at 72°C for 45 cycles, and 7 min at 72°C for a final extension. The PCR products were separated on 1.5% agarose gels buffered with 0.5× TBE, and detected by staining with ethidiumbromide.

Nucleotide sequences data analysis

DNA sequences were assembled using DNASTAR software (DNASTAR 1994) and aligned using ClustalX (Thompson et al. 1997). All sequences were submitted to GenBank. For each data set, maximum parsimony (MP) analysis was performed using a branch-and-bound search using PAUP* 4.0b5 (Swofford 1998) to construct phylogenic trees. According to the phylogenetic study of Rhizophoraceae (Zhong et al. 2000; Shi et al. 2002), we selected *Bruguiera gymnorrhiza* as outgroup in this study. Strict consensus trees were constructed from all most-parsimonious trees. Bootstrap analyses were carried out with 1,000 replicates using TBR branch-swapping of the heuristic search with random taxon addition (Felsenstein 1985). Characters were assigned equal weights at all nucleotide positions (Fitch 1971). Gaps were treated as missing data. Sequence divergences were estimated using *P* distance (Saito and Nei 1987).

ISSR data analysis

ISSR bands were scored as present (1) or absent (0) for each DNA sample excluding the smeared and weak ones. The banding patterns were analyzed as phenetic data, or as genetic data for tests of population genetic diversity and genetic architecture. As genetic data, the binary data matrix was input into POPGENE (Yeh et al. 1997), assuming Hardy–Weinberg equilibrium. The following indices were estimated: the percentage of polymorphic loci (*P*), the expected heterozygosity (He) (Nei 1973), total heterozygosity (Ht), heterozygosity value within populations (Hs), and the proportion of genetic diversity between populations (G_{ST}). Bootstrap analysis (1,000 duplicates) and Neighbor-Joining diagrams were constructed on genetic distances (Nei 1978) among populations using RAPDDIST (Black 1995) and the NEIGHBOR program in PHYLIP 3.5C (Felsenstein 1993). Gene diversity statistics were further calculated for the three geographical regional groups identified by the cluster analysis, i.e., EIO, SCS, and the coast of NA.

For phenetic analyses, analysis of molecular variance (AMOVA) was performed using AMOVA Version 1.55 (Excoffier 1993) to determine whether genetic variation was distributed according to current boundaries within *Ceriops*, or, alternatively, whether genetic variance is partitioned geographically as might be the case if

morphospecies are not monophyletic groups. If the results support the morphospecies are independent evolutionary lineages, most genetic variance within our samples should be partitioned among groups of populations defined by morphology (see Nice et al. 2005). A second AMOVA were arranged to calculate the genetic variation distribution among regions, among populations within a region, and within populations. In order to test for the correlation between genetic and geographical distances among populations, a Mantel test was performed using NTSYSpc V2.02j (Rohlf 1998) by computing 5,000 permutations.

Results

DNA sequences

The total length of *G3pdh* of all *Ceriops* individuals was 862 bp (GenBank accession no. EF423375, EF423376, EF423383, EF423384, EF423385, and EF423386). Excluding a 18-bp deletion occurring only on the haplotype of *C. tagal*, a total of 41 polymorphic sites (4.8% of the total length) were detected, which consist of 38 point mutations and 3 indels. Of the 812-bp *trnV-trnM* aligned sequences of *Ceriops*, four long fragments of indels (7, 19, 20, and 21 bp, respectively) were detected. A total of 28 *trnV-trnM* sites were polymorphic (3.4% of the total sequence) with 20 point mutations and 8 indel sites (excluding the long indel fragments) (GenBank accession no. EF423377, EF423378, EF423379, F423380, and EF423381). Among species, the lowest nucleotide divergence was between *C. tagal* and *C. australis* (estimates of pairwise divergence between them is 13, and 1, in *G3pdh* and in *trnV-trnM*, respectively, with the exception of indel fragments).

A total of six haplotyes of *G3pdh* and five haplotyes of *trnV-trnM* were recognized among the three species. No haplotype diversity was found within populations. Only *C. decandra* was detected having more than one haplotype from each DNA sequence data set. In *C. decandra*, a total of four *G3pdh* haplotypes (the estimates of pairwise distances among them varied from 2 to 15) and three *trnV-trnM* haplotypes (the value of pairwise divergence among them ranged from 3 to 16) were detected. Both *G3pdh* and *trnV-trnM* data of *C. decandra* identified three groups in correspond to the geographical region EIO, SCS, and NA (Fig. 2a, b).

ISSRs

Genetic diversity and phylogenetic analysis of the genus

A total of 179 loci and 201 genotypes were presented from the 11 ISSR primers for the three species across 385 individuals of 30 populations. The size of the ISSR fragments varied from 0.2 to 1.4 kb. Of the 179 loci surveyed, 159 were polymorphic in at least one of the sampled populations. The number of unique loci of each species detected in *C. decandra*, *C. tagal*, and *C. australis* was 21, 4, and 2, respectively. The total ISSR diversity (Ht) of all individuals from the three species is 0.308 (data not shown). The expected heterozygosity of each population (He) was presented in Table 1. Analyses of variance (ANOVA) indicated the value of expected heterozygosity was independent of population size ($P > 0.05$, data not shown).

The Neighbor-Joining dendrogram revealed three distinct groups in correspond to *C. decandra*, *C. tagal*, and *C. australis* (Fig. 2c). AMOVA revealed that most of the variation (56.24%) was held between species (Table 2). All components of molecular variance were highly significant ($P < 0.001$).

Genetic differences and population structure within species

Of the species *C. decandra*, 11 ISSR primers produced a total of 164 loci and 78 ISSR genotypes across the 189 individuals from 13 populations. At the species level, the percentage of polymorphic loci (*P*) was 79% (Table 3). Ht was 0.270 compared with the heterozygosity value of 0.028 within populations (Hs), and the proportion of genetic diversity between populations (G_{ST}) was 0.897.

In the case of *C. tagal*, a total of 136 loci and 88 ISSR genotypes were presented across all 152 individuals of 14 populations. At the species level, the value of *P* was 54%. The estimate of Ht in *C. tagal* was 0.118, whereas the heterozygosity within populations (Hs) was 0.023, with a G_{ST} value of 0.802 (Table 3).

For the three populations of *C. australis*, a total of 117 loci and 35 ISSR genotypes were detected across all 44 individuals of the three populations. The percentage of polymorphic loci was 29% at the species level. The Ht, Hs, and G_{ST} of *C. australis* is 0.089, 0.053, and 0.402, respectively (Table 3).

Three major groups in correspond to the geographical regions SCS, EIO, and NA were revealed by cluster analysis in *C. decandra* and *C. tagal* with the bootstrap values varied from 57.1 to 100% (Fig. 2c). Mantel test indicated no statistically significant correlation between pairwise genetic distance and corresponding geographic distance among populations within species (data not shown).

AMOVA revealed that significant population differentiation for the two widespread species, *C. decandra* and *C. tagal* (PHI_{ST} = 0.898, and 0.790, respectively) (Table 2). Most of genetic variation (75.83% for *C. decandra*, and 46.25% for *C. tagal*) was partitioned among regions (SCS, EIO, and NA), with a small part of genetic variation accounting for among populations within regions and among individuals within populations. For the endemic species

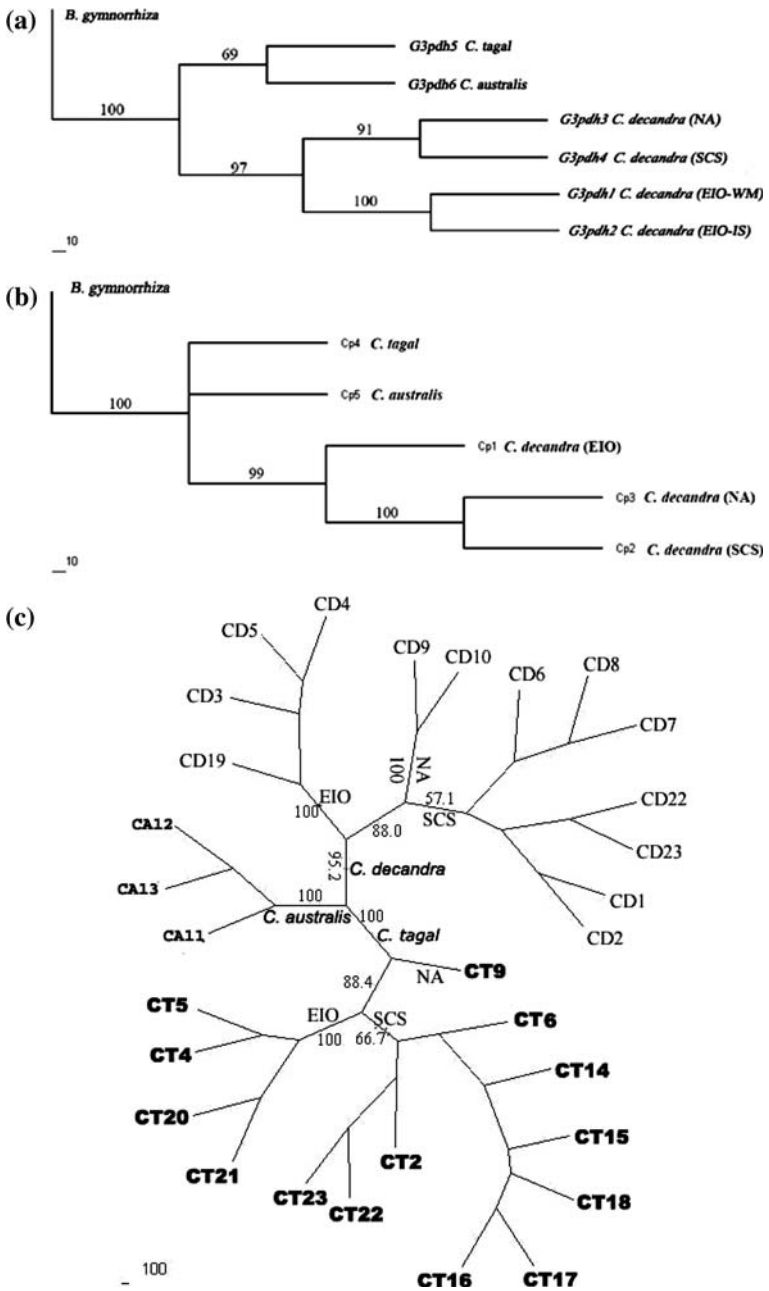

Fig. 2 Trees of *Ceriops* constructed by molecular data. (**a**) The most parsimony tree inferred from sequences of *G3pdh*; (**b**) the strict consensus tree inferred from sequences of *trnV-trnM*. *NA* North Australia, *SCS* South China Sea, *EIO* East Indian Ocean, *WM* West Malay Peninsula, *IS* India/Sri Lanka area; (**c**) Neighbor-Joining tree obtained with 1,000 bootstraps on Nei's genetic distance (1978) (see Table 1 and Fig. 1 for population locations). Bootstrap values are reported as percentages

C. australis, 43.24% of the total variation was accounted for among populations, with a variation component of 56.76% among individuals within a population. All components of molecular variance were highly significant ($P < 0.001$).

Discussion

DNA sequence and ISSR markers

The detection of high levels of polymorphism (P = 79, 54, and 29% in *C. decandra*, *C. tagal*, and *C. australis*, respectively) makes ISSR analysis a powerful tool for assessing genetic diversity within species. Although only *C. decandra* was detected containing more than one haplotype from DNA sequences, there were still some highlights contributed to this study. The geographical groups of the chloroplast haplotypes within *C. decandra* is correlated to that of nucleotid*e* haplotypes. The spatial pattern of genetic structure inferred from nuclear sequences (*G3pdh*) of *C. decandra* was as well consistent with that revealed by the nuclear genome data (ISSRs).

Phylogenetics of the genus

White (1926) observed that in Australia and Papua New Guinea there was a form of *C. tagal* whose propagules had

Table 2 Results from hierarchical AMOVA of *Ceriops* populations

A. Source of variation	*df*	Variance component	% Of total variance	*P*-value	PHI_{ST}
Total of three species					0.924
Among species	2	20.38	56.24	<0.001	
Among populations within species	27	13.69	37.78	<0.001	
Within populations	355	2.17	5.98	<0.001	
B. Source of variation	*df*	Variance component	% Of total variance	*P*-value	PHI_{ST}
C. decandra					0.898
Among regions (SCS, EIO, and NA)	2	24.91	75.83	<0.001	
Among populations within regions	10	5.49	16.72	<0.001	
Within populations	176	2.45	7.45	<0.001	
C. tagal					0.79
Among regions (SCS, EIO, and NA)	2	4.57	46.25	<0.001	
Among populations within regions	11	3.63	36.69	<0.001	
Within populations	138	1.69	17.06	<0.001	
C. australis					0.432
Among regions (SCS, EIO, and NA)	–	–	–	–	
Among populations	30.113	1.95	43.24	<0.001	
Within populations	2.567	2.57	56.76	<0.001	

(A) Results grouping populations by morphological species. (B) Results grouping populations by regions determined by population clustering analysis (see text, Fig. 2c)

Table 3 Comparison of genetic diversity estimates in *Ceriops* between different groups of populations as determined using ISSR markers

Parameters	Total	Region		
		EIO	SCS	NA
C. decandra				
% of polymorphic loci (*P*)	79	34	34	8
Total gene diversity (Ht)	0.270 ± 0.040	0.106 ± 0.028	0.103 ± 0.029	0.028 ± 0.010
Within population (Hs)	0.028 ± 0.002	0.041 ± 0.007	0.022 ± 0.002	0.022 ± 0.006
Between populations (Dst)	0.242 ± 0.038	0.065 ± 0.022	0.081 ± 0.027	0.006 ± 0.004
Coefficient of gene differentiation (G_{ST})	0.897	0.617	0.784	0.222
C. tagal				
% of polymorphic loci (*P*)	54	28	29	15
Total gene diversity (Ht)	0.118 ± 0.024	0.084 ± 0.025	0.070 ± 0.019	0.049 ± 0.012
Within population (Hs)	0.023 ± 0.002	0.036 ± 0.006	0.015 ± 0.001	0.049 ± 0.012
Between populations (Dst)	0.094 ± 0.022	0.048 ± 0.019	0.055 ± 0.017	-
Coefficient of gene differentiation (G_{ST})	0.802	0.575	0.787	-
C. australis				
% Of polymorphic loci (*P*)	29			
Total gene diversity (Ht)	0.089 ± 0.025			
Within population (Hs)	0.053 ± 0.011			
Between populations (Dst)	0.036 ± 0.014			
Coefficient of gene differentiation (G_{ST})	0.402			

smooth, terete hypocotyls rather than the angled or ribbed hypocotyls typical of *C. tagal*, and described the atypical form as a variety of *C. tagal*, *C. tagal* (Perr.) C. B. Robinson var. *australis* C. T. White. However, in a more recent systematic revision, the evidence of distinct allozyme phenotypes and the reproductive isolation between *C. tagal* and *C. tagal* var. *australis* confirmed that the variety should be recognized as a species distinct from *C. tagal* (Ballment et al. 1988). In our study, two monophyletic clades corresponding to the *C. tagal* and *C. australis* were supported by

Neighbor-Joining dendrogram inferred from the ISSR data (Fig.2c). AMOVA analysis also revealed that most of the variances of these two species were accounted for between species (Table 2). In addition, no haplotype was shared by *C. tagal* and *C. australis* (Fig. 2a, b). All of these results in our study supported *C.tagal* and *C. australis* should be treated as separate groups.

Genetic diversity and population structure within species

At the population level, the expected heterozygosity (He) of *Ceriops* was low inferred from ISSRs (Table 3), and from DNA sequences as well (no haplotype diversity and no nucleotide diversity within populations were detected). Such low population genetic diversity was also presented in the previous study of *C. tagal* from South China and Thailand (Ge and Sun 2001), and in some other mangrove species, e.g., *Aegiceras corniculatum*, *B. gymnorrhiza*, *Avicennia marina* (Ge and Sun 1999; Ge 2001), using the ISSR technology. In this study, the expected heterozygosity varied widely among populations of each species, but was not related to any obvious characteristics of the populations, such as their isolation at the margins of the range of the species or sample size (Table 1). Although species of *Ceriops* show self compatibility, their pollination may favor outcrossing (Tomlinson 1986). Such a mixed mating model has existed in some other mangrove species of Rhizophoraceae (Lowenfeld and Klekowski 1992; Klekowski et al. 1994; Sun et al. 1998; Ge 2001). Variation between populations in outcrossing rates has commonly been revealed in mixed mating species (Schoen 1982). Low-gene diversity in this study may also arise from population substructure or founder events, in which high level of inbreeding have led to demes of genetically similar individuals (see Tan et al. 2005). The higher genetic variation inferred from isozyme (S. Huang, personal communication) and high cpDNA haplotype diversity (Liao et al. 2006) in *C. tagal* may be due to different molecular markers being used and different sampling methods.

At the species level, both of the ISSR data and DNA sequences revealed the greatest diversity within *C. decandra* of the genus. As Hamrick et al. (1991) suggested, several factors are important in determining levels of genetic diversity. Geographic range is strongly associated with the level of variation maintained at the species level. Generally endemic species have lower genetic diversity than widespread species. In contrast to the restricted sampling of the endemic species *C. australis*, *C. decandra*, and *C. tagal* were both sampled covering a wide geographic range throughout the IWP in this study. Other factors such as breeding systems, vegetative reproduction, dispersal pattern, sample size, etc., also significantly influence the genetic diversity of a species. Considering the geographic sampling range (*C. tagal* have a wider sampling range than *C. decandra*, see Fig. 1), the sample size (152 individuals from 14 population in *C. tagal* versus 189 from 13 population in *C. decandra*), and the similar life history and biological characteristics, it is interesting that *C. tagal* exhibited much lower genetic diversity than *C. decandra*. DNA sequences may give some highlight on time scale. No haplotype diversity was detected in *C. tagal*, whereas four *G3pdh* haplotypes and three *trnV-trnM* haplotypes (the diversity between these haplotypes is also high) were found in *C. decandra*. The species *C. tagal* which seems to be able to survive in a wider range of climate may not have been splitted as much as *C. decandra* in different refugia, and thus the separation of the different *C. tagal* groups might be more recent.

Significant population genetic structure was detected in both *C. decandra* and *C. tagal*. Both genetic data and phenetic data (AMOVA) indicated significant inter-population variation in genetic structure. The fixation index (G_{ST} = 0.897, and 0.802 in *C. decandra*, and *C. tagal*, respectively) was close to the partition of genetic variance (PHI_{ST}) by AMOVA (Table 2), based on similarities of ISSR fingerprints. A dendrogram based on genetic distances of ISSR revealed three major clades corresponding to three well-delineated geographical regions (i.e., the EIO, SCS, and NA) within the distribution of *C. decandra* and *C. tagal*. Similarly, the *G3pdh* MP tree and the *trnV-trnM* strict consensus tree displayed the same three geographical groups in *C. decandra*. Furthermore, hierarchical AMOVA revealed that most of the total variation (76% in *C. decandra*, and 46% in *C. tagal*) was accounted for by differentiation between regions. The separation of the SCS populations from the EIO populations in this study is consistent with the result of previous studies on *C. tagal* within the Malay Peninsula using cpDNA sequences and ISSRs (Liao et al. 2006; Ge and Sun 2001). Generally, the present distribution of genetic structure is influenced by both natural history and evolutionary history (Hamrick and Godt 1996). For plants whose seeds are passively dispersed, the seed dispersal ability of a species is one of the major determinants accounting for population differentiation (Dodd et al. 2002). *Ceriops* are viviparous mangroves, whose propagules are about 10–20 cm long (Tomlinson 1986). Their viviparous seedlings are buoyant, presumably capable of long-distance dispersal by ocean currents. However, field experiments suggested their propagule dispersal is apparently limited by surface currents, the presence of suitable environmental conditions, and longevity of propagules during dispersal (Clarke et al. 2001; see Ge and Sun 2001). In addition, a field experiment has

revealed very limited dispersal capability on *C. australis* propagules (only 9% of marked propagules were found more than 3 m from the parent trees) (McGuinness 1997). Thus, the long-distance dispersal of propagules is rather limited in *Ceriops* species. The geographic history is another factor that may have influenced genetic differentiation in this area (Ge and Sun 2001). During the Pleistocene, sea levels fluctuated repeatedly, dropping as low 200 m below, and occasionally rising above, present levels. The genetic differentiation was thought to occur when gene flow was restricted, probably at times of lowered sea level when land connected much of South East Asia, New Guinea and Australia, almost closing the sea connecting between the Indian and Pacific Oceans (Benzie 1998). Even today, the Indian Ocean is connected with SCS only through the Strait of Malacca, which make it an effective barrier to some mangrove species between the Pacific and Indian Oceans (Ge and Sun 2001; Tan et al 2005). Within-region differentiation seems to be high in the EIO and SCS, but these areas need further study. With more extensive sampling, especially in Australia and Indonesia, and a more efficient DNA sequence marker will provide more information to infer the phylogeographic pattern of the *Ceriops* species.

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