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ORIGINAL PAPER

Phylogeography of *Ceriops tagal* (Rhizophoraceae) in Southeast Asia: the land barrier of the Malay Peninsula has caused population differentiation between the Indian Ocean and South China Sea

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Abstract The genetic structure of mangrove species is greatly affected by their geographic history. Nine natural populations of *Ceriops tagal* were collected from Borneo, the Malay Peninsula, and India for this phylogeographic study. Completely different haplotype compositions on the east versus west coasts of the Malay Peninsula were revealed using the *atpB-rbcL* and *trnL-trnF* spacers of chloroplast DNA. The average haplotype diversity (Hd) of the total population was 0.549, nucleotide diversity (θ) was 0.030, and nucleotide difference (π) was 0.0074. The cladogram constructed by the index of population differentiation (G_{ST}) clearly separated the South China Sea populations from the Indian Ocean populations. In the analysis of the minimum spanning network, the Indian Ocean haplotypes were all derived from South China Sea haplotypes, suggesting a dispersal route of *C. tagal* from Southeast Asia to South Asia. The Sunda Land river system and surface currents might be accountable for the gene flow directions in the South China Sea and Bay of Bengal, respectively. The historical geography not only affected the present genotype distribution but also the evolution of *C. tagal*. These processes result in the genetic differentiation and the differentiated populations that should be considered as Management Units (MUs) for conservation measurements instead of random forestation, which might lead to gene mixing and reduction of genetic variability of mangrove species. According to this phylogeographic study, populations in Borneo, and east and west Malay Peninsula that have unique genotypes should be considered as distinct MUs, and any activities resulting in gene mixing with each other ought to be prevented.

Keywords *Ceriops tagal* · *atpB-rbcL* · *trnL-trnF* · Phylogeography · Land barrier · Management Units

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Introduction

Mangroves are widely distributed in tropical and subtropical coastal regions (Duke 1992). Mangroves not only act as nurseries for local fisheries and aquaculture, but also protect the coasts. Most mangroves have viviparous propagules which are buoyant and presumably capable of dispersal by ocean currents. The population genetic structure of these species is likely to be affected by the currents in the ocean. Previous studies indicated that some geographical differentiation may be caused by land barriers (Dodd and Rafii 2002) or past land barriers (Duke et al. 2002). In a genetic study of *Kandelia* using mitochondrial DNA and chloroplast DNA markers, it was found that a historical vicariance event might have influenced the genetic structure of the populations around the South China Sea (Chiang et al. 2001). During the Pleistocene, approximately 0.01–0.11 million years ago [Mya], due

to global glaciation, sea levels were about 100–120 m lower than current levels in South China Sea, and the Sunda Land, an exposed land ridge, separated the Pacific and Indian Oceans (Voris 2000). Even today, the Malay Peninsula is still an effective barrier to some mangrove species between the Pacific and Indian Oceans (see Ge and Sun 2001; Tan et al. 2005).

The phylogeny of the Rhizophoraceae (Zhong et al. 2000; Shi et al. 2002) and other mangrove families (e.g., the Sonneratiaceae, Shi et al. 2000) are well understood based on functional genes, and some population genetic studies have been carried out: Maguire et al. (2000) used microsatellites to characterize the genetic structure of *Avicennia marina*; Dodd and Rafii (2002) used AFLPs to study the gene diversity and population genetic structure of *A. germinans*; Sun et al. (1998) used isozymes to distinguish and portray the population genetic structure of *Kandelia candel* (this species was classified as *K. obovata* by Sheue et al. [2003]) in Hong Kong and its mating system; while Ge and Sun (2001) and Tan et al. (2005) used ISSR to explore the genetic structure of *C. tagal* and *C. decandra*, respectively. However, those researchers only discussed the ''population genetic structure'' instead of the evolutionary history of those species. In only a few instances was the topic of ''phylogeography'' in Southeast Asia mentioned; for example, Chiang et al. (2001) used chloroplast and mitochondrial DNA sequences to study the population differentiation and phylogeography of *K. candel*, after which, this species was examined and identified as a new species, *K. obovata*, in the northern South China Sea and East China Sea by Sheue et al. (2003). Duke (1995) proposed a theory of ''center of origin'' of mangroves and combined this theory with the idea of the ''center of diversity'' of mangroves. Duke et al. (2002) and Duke (1995) used *Avicennia* and*Rhizophora* as model genera to draw an evolutionary pathway of mangroves. Herein, we present a scenario to explore the evolutionary history of *Ceriops tagal* in South and Southeast Asia.

Ceriops tagal (Perr.) C. B. Robinson (Rhizophoraceae) typically grows in inner mangroves, and is geographically widespread from East Africa through India and Malaysia to South China (Tomlinson 1986). The origin of *Ceriops* can be traced back to ~132 Mya since the divergence of the Rhizophoraceae (Zhong et al. 2000). According to Zhong et al. (2000) and Shi et al. (2002), *Ceriops* is the basal lineage of the Rhizophoraceae and was grouped with *Kandelia, Bruguiera*, and *Rhizophora* in studies of functional gene markers. The effects of selection pressures may have influenced the accuracy of the phylogeny through the elimination or retention of genes. Thus, neutral markers are necessary for the study of the relationship between species and their geographical histories. An example of such markers is chloroplast DNA, which can be considered as a single unit of inheritance and not subject to recombination (Schaal et al. 1998; Newton et al. 1999). Many phylogeographic studies in plants have taken advantage of this marker, and assessed the variations in chloroplast DNA. Two of the most commonly used neutral genetic markers in population genetic and phylogeographic studies are the chloroplast *trnL-trnF* and *atpB-rbcL* spacers, which were also used for constructing the phylogeography of *Ceriops tagal* in this study.

From the studies of phylogeography, a population (or a set of populations) that ''is reciprocally monophyletic'' and ''shows significant divergence of allele frequencies'' could be defined as an Evolutionarily Significant Unit (ESU) (Ryder 1986; Fraser and Bernatchez 2001). ESUs were developed to provide an objective approach to prioritizing units for protection below taxonomic levels (Ryder 1986). Moritz (1994) defined ''Management Unit (MU)'', which is the significant divergence of alleles/haplotypes. MUs could be used to assist the development of conservation and sustainable use of plant species (Newton et al. 1999) without the controversial determination of how much phylogenetic difference in alleles is enough to be used in practice. From previous phylogeographic studies, the MUs (and/or ESUs) of mangrove species can clearly be defined and used as guidance to plant conservation, especially for informing the transfer of germplasm within and between regions, as can serve in the reforestation or restoration activities (Newton et al. 1999).

We report here the haplotype distribution and genetic variation of *C. tagal* in Southeast Asia and the possible evolutionary history of *C. tagal* between the South China Sea and Bay of Bengal (Indian Ocean). We focus on the differentiation between populations in the South China Sea and Bay of Bengal, especially on both sides of the Malay Peninsula. Genetic information should help provide evidence of the phylogeography of this species.

Methods

Plant materials

Leaves were obtained from 70 trees of *C. tagal* from four populations in the Indian Ocean (West Bengal [WB, $n = 2$], Ranong [RN, $n = 9$], Phang-Nga [PN,

n = 9], and Phuket island [PK, n = 10]) and five in the South China Sea (Tumethong Amphur Patew [PT, n = 8], Thungkra-Swi Amphur Sawi [SW, n = 10], Surat Thani [ST, n = 8], Kuching [KC, n = 6], and Tuaran [TR, n = 8]) (Table 1, Fig. 1). All of the trees were randomly sampled along the rivers and were at distances at least 20 m from each other. The leaves were dried with silica gel immediately after sampling to preserve the DNA.

DNA extraction and amplification

The dried foliage was ground to a powder using liquid nitrogen and then extracted with a plant DNA extraction mini kit (Viogene). The DNA was dissolved in ddH_2O and stored at −20°C.

PCR amplification was performed in 10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 7% DMSO, 200 nM primers, 1 unit of Taq polymerase, and ~20 ng genomic DNA per 20μl reaction. The *atpB-rbcL* spacer and *trnL-trnF* spacer of chloroplast DNA (cpDNA) were used in this study. The forward and reverse primers of *atpB-rbcL* spacer were designed in this research (the primers were designed from cloned and sequenced fragments of *atpB* gene and *rbcL* gene of *C. tagal*): atpB-ct, 5′-CCAGAAGTAGTCGGATT GAT-3′; and rbcL-ct, 5′-AGTTACTCGGAATGC TGCCA-3′, and the annealing temperature was optimized at 55°C. The *trnL-trnF* primer referred to Taberlet et al. (1991): IGS-e, 5′-GGTTCAAGTCCCTCT ATCCCC-3′; and IGS-f, 5′-ATTTGAACTGGTGA-CACGAG-3′. The annealing temperature of *trnL-trnF* was optimized at 60°C. The PCR cycles were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 40 s, at the annealing temperature for 40 s, at 72°C for 1.5 min, and at 72°C for a final 7 min extension. The amplified DNA was checked using a 1% agarose gel with ethidium bromide and directly sequenced. Both strands were sequenced using the Big-Dye Terminator Sequencing Kit (PE Applied Biosystems) with the same primers used for PCR amplification. Double-stranded DNA was cycle sequenced

Table 1 Populations of *Ceriops tagal* sampled

Population	Abbreviation	Longitude	Latitude	Country	Sea area
West Bengal	WB	24°03′ N	88°07′ E	India	Indian Ocean
Ranong	RN	09°55′ N	98°37′ E	Thailand (Malay Peninsula)	Indian Ocean
Phang-Nga	PN	08°24′ N	98°30′ E	Thailand (Malay Peninsula)	Indian Ocean
Phuket island	PK	08°11′ N	98°17′ E	Thailand (Malay Peninsula)	Indian Ocean
Tumethong Amphur Patew	PT	10°00′ N	99°52′ E	Thailand (Malay Peninsula)	S. China Sea
Thungkra-Swi Amphur Sawi	SW	10°15′ N	99°05′ E	Thailand (Malay Peninsula)	S. China Sea
Surat Thani (Korn Nan)	ST	09°07′ N	99°20′ E	Thailand (Malay Peninsula)	S. China Sea
Kuching, Sarawak	KC	01°40′ N	111°12′ E	Malaysia (Borneo)	S. China Sea
Tuaran, Sabah	TR	06°13′ N	116°12′ E	Malaysia (Borneo)	S. China Sea

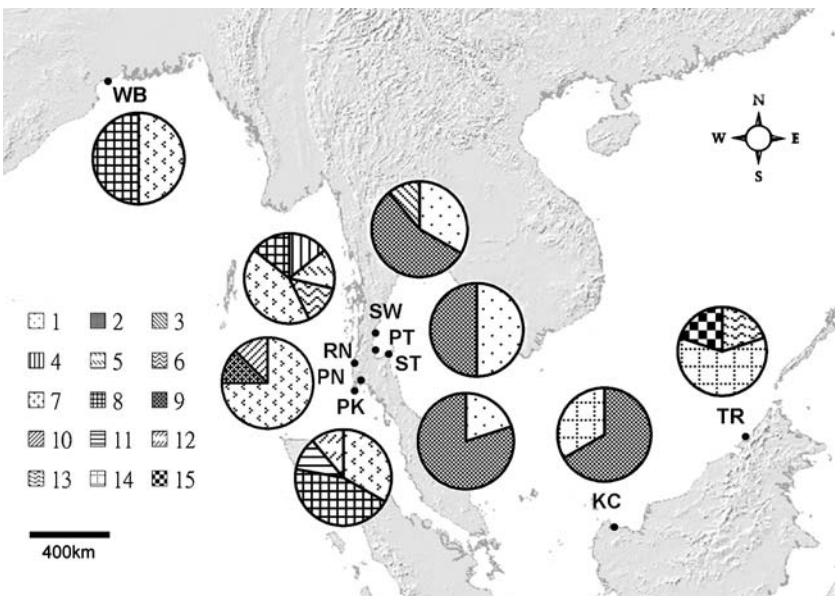

Fig. 1 *atpB-rbcL* + *trnL-trnF* haplotype distribution of *Ceriops tagal* in Southwest Asia

using standard protocol and run on ABI 377 automated sequence analyzer (PE Corp.). These sequences are deposited in GenBank under accession numbers DQ145943-DQ145963.

Data analysis

DNA sequences were checked using the naked eye with the assistance of DNASTAR software, and aligned using ClustalX (Thompson et al. 1997). After alignment, the genetic variability and genetic differentiation between each population were estimated with the DnaSP software (Rozas et al. 2003). We used the haplotype (gene) diversity (Hd, Nei 1987), the pairwise difference of nucleotide (π, Nei 1987), and the nucleotide diversity (θ, Nei, 1987) to reveal the genetic diversity of *C. tagal* in every population. The index G_{ST} (Nei 1987), which is a coefficient of gene differentiation, was estimated from the sequence differences, to draw an UPGMA tree using MEGA 3 (Kumar et al. 2004). Because of the long indel fragments, we treated gaps as the fifth character and took every gap fragment as an evolutionary event in the network construction (Fig. 2). The Neighbor-joining (NJ) method and Bayesian inferences (BI) were used to construct the phylogenetic trees. NJ was conducted using MEGA 3. In the NJ method, the gaps were treated as another character and conducted using p-distances for preventing needless weighting of long indel fragments. Confidence in the clades was tested by bootstrapping with 1000 replicates on the 50% majority rule trees. The BI used MrBayes 3.1 (Ronquist and Huelsenback 2003) with the following settings: Each indel was considered as the fifth character, and every substitution and indel event was regarded as a single and equal evolutionary change, such that the setting of one was chosen for N_{st} (the number of substitution types) which assumes equal substitution rates at all sites; the model for among-site variation was ''invgamma'' (gamma-shaped rate variation with a proportion of invariable sites); the number of generation (N_{gen}) was 10 million; the sample frequency was 100 steps; the temperature parameter for heating the chains was 0.2; and the burn-in was 100 samples. In the network construction, the minimum spanning network method was used with the MINSPNET software (Excoffier and Smouse 1994). Every indel fragment was treated as an evolutionary event (Fig. 2), and pairwise differences in the matrix of number of mutations were constructed using MEGA 3; then every possible link was calculated among the haplotypes.

Results

Nucleotide sequence polymorphism

The partial sequences of the *atpB-rbcL* spacer used in this study were 917 base pairs (bp) (consensus length) after alignment, and ranged from 801 to 855 bp. The genetic diversity (Hd) of the total population was 0.667 (eight haplotypes), the nucleotide diversity (θ) was 0.0068, and the average nucleotide differences (π) was 0.0031. In DNA sequencing of the *trnL-trnF* spacer, because of the DNA structure (two long fragments of poly T), only 266 bp partial sequences (consensus length) could be used, and ranged from 222 to 264 bps. Among these 266 bp sequences, Hd was 0.671 (13 haplotypes), θ was 0.1259, and π was 0.0252. After combining both sequences, Hd was 0.549 (15 haplotypes), θ was 0.0299, and π was 0.0074. Among these populations, WB has the highest haplotype diversity (Hd = 1.000) since its two haplotypes are different in *trnL-trnF* spacer. RN had the second highest haplotype diversity (Hd = 0.857), followed by TR (Hd = 0.750). With more complete sampling in Malay Peninsula and Borneo have, would confirm if RN and TR are more diverse than other populations. The detailed population genetic variations are displayed in Table 2. Overall, the genetic variability of the *trnL-trnF* spacer

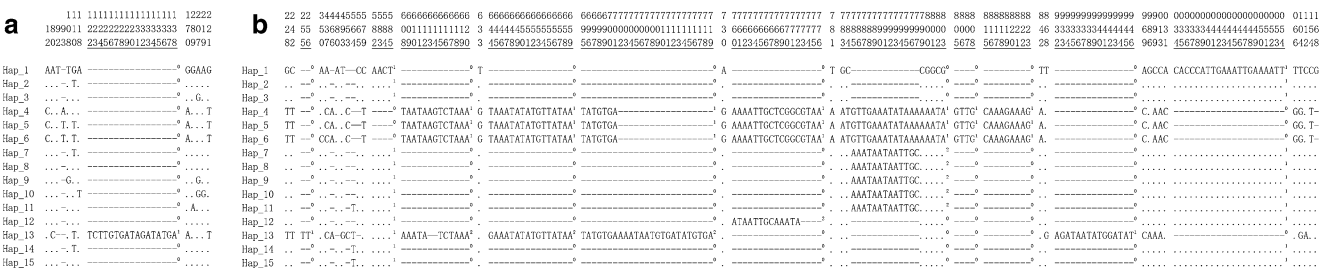

Fig. 2 Variable sites of (**a**) *trnL-trnF* (sites 12–221) and (**b**) *atpB-rbcL* (sites 228–1168) spacers of *Ceriops tagal*. Note that most of the variable sites are long indel fragments, which are underlined in the site number. The superscripts of the long indel fragments are the coding numbers

Table 2 Genetic variability within populations detected by the *atpB-rbcL* and *trnL-trnF* spacer sequences of cpDNA. Note that π and θ were calculated excluding gaps because these two parameters were estimated by the "nucleotide substitutions" per site between two sequences

Population	*atpB-rbcL* + *trnL-trnF*					*atpB-rbcL*					*trnL-trnF*				
	n	*H*	Hd	π	θ	*n*	*H*	Hd	π	θ	*n*	*H*	Hd	π	θ
PT	8	2	0.571	0.56	0.38	8	1	0	0	0	8	2	0.571	2.56	1.73
SW	9	3	0.639	0.76	0.72	9	1	0	0	0	10	3	0.600	3.29	3.17
RN	7	5	0.857	19.99	14.55	7	3	0.667	21.42	15.56	9	5	0.861	15.70	13.20
PN	9	3	0.464	1.55	1.86	9	1	0	0	0	9	4	0.583	108.11	177.34
PK	9	4	0.750	0.71	0.72	10	3	0.378	0	0	9	3	0.639	3.24	3.30
ST	5	2	0.400	0.39	0.47	8	2	0.250	2.89	4.46	6	3	0.600	39.76	51.06
TR	5	3	0.700	5.99	7.19	8	2	0.200	2.89	4.46	6	3	0.733	13.51	11.84
KC	3	2	0.667	0	0	3	2	0.667	0	0	6	4	0.800	8.67	7.86
WB	2	2	1.000	0.96	0.96	2	1	0	0	0	2	2	1.000	4.48	4.48
Total	56	15	0.549	7.35	29.89	64	8	0.667	3.07	6.84	65	13	0.671	25.15	125.91

n is the sample size of the marker used; *H* is the number of haplotypes; Hd is the gene diversity (Nei, 1987); π is the average number of nucleotide differences per site between two sequences (Nei, 1987); and θ is the nucleotide diversity estimated by the total number of mutations (Nei, 1987)

Population codes are given in Table 1

was higher than that of the *atpB-rbcL* spacer. Most of the variable sites were indels, especially the long-fragment indels in the *atpB-rbcL* spacer.

Haplotype distribution among populations

There were different haplotype compositions in the South China Sea and Indian Ocean: six haplotypes (Hap 1, 2, 3, 13, 14, and 15) in the South China Sea and nine haplotypes (Hap 4, 5, 6, 7, 8, 9, 10, 11, and 12) in the Indian Ocean. On the eastern Malay Peninsula, only three haplotypes were detected, and Hap 2 was the dominant one. Hap 2 was also detected in the Kuching (KC) population. KC had another haplotype, Hap 14, which was the dominant haplotype at Tuaran (TR). Hap 13 and 14 were endemic haplotypes in TR. They reflected differences in *C. tagal* between Borneo and the Malay Peninsula. In addition, Hap 1 was an endemic haplotype in eastern Malay Peninsula populations, and Hap 3 was only found in the Sawi (SW) population.

Hap 7 was the dominant haplotype and was detected in all Indian Ocean populations. Hap 8 was the second dominant haplotype in the Indian Ocean and was shared with the Ranong (RN), Phuket island (PK), and West Bengal (WB) populations. RN had five haplotypes and had the highest haplotype diversity (Hd = 0.857). Although only two samples of the WB population were evaluated, each had a different haplotype in the *trnL-trnF* spacer.

Phylogeographic relationships

Sequence differences of the major haplotypes of the South China Sea and Indian Ocean, Hap 2 and Hap 7, respectively, were distinguished by one indel fragment. The phylogenetic trees constructed by both NJ method (*atpB-rbcL* spacer, *trnL-trnF* spacer and *atpB-rbcL* + *trnL-trnF* spacers are shown in Fig. 3a, b, and c, respectively) and Bayesian inference (Fig. 4, only the *trnL-trnF* and *atpB-rbcL* combined tree is shown) had similar topologies (same grouping patterns) in that Hap 1, 2, 3, 7, 8, 9, 10, 11, 12, 14, and 15 were grouped together and were not well resolved. Long branches of Hap 4, 5, 6, and 13 revealed high divergences from other haplotypes. The highly divergent haplotypes were all detected in a single population: Hap 13 was an endemic haplotype in Tuaran, Borneo, and Hap 4, 5, and 6 were endemic to Ranong, Thailand. The NJ tree of *atpB-rbcL* spacer, in general, match the geographic distribution pattern except Hap 13 (endemic haplotype of TR) and Hap 4, 5, and 6 (endemic to RN) (Fig. 3a). The phylogenetic tree of *trnL-trnF* spacer, however, does not match the geographic distribution pattern (Fig. 3b), but suggests the evolutionary routes, which is consistent with the BI (Fig. 4) and network (Fig. 5) results: First, Hap 2 might have originated from Malay Peninsula, ended up in Borneo, and derived into Hap 14, when the haplotype frequency distribution is considered; second, Hap 1 might be the original haplotype which derived to Hap 2, 3, 8, 12, and 15 (Fig. 5); third, in the Indian Ocean populations, most of the haplotypes were derived from East Malay Peninsula populations, for instance, Hap 7 might be derived from Hap 2, and Hap 8 from Hap 1 (Fig. 5). In the analysis of the minimum spanning network, Hap 1 was the ancestral haplotype. This haplotype is widely distributed in eastern Malay Peninsula populations. The most-dominant haplotype, Hap 2, in the eastern Malay Peninsula might be derived from Hap 1. Furthermore,

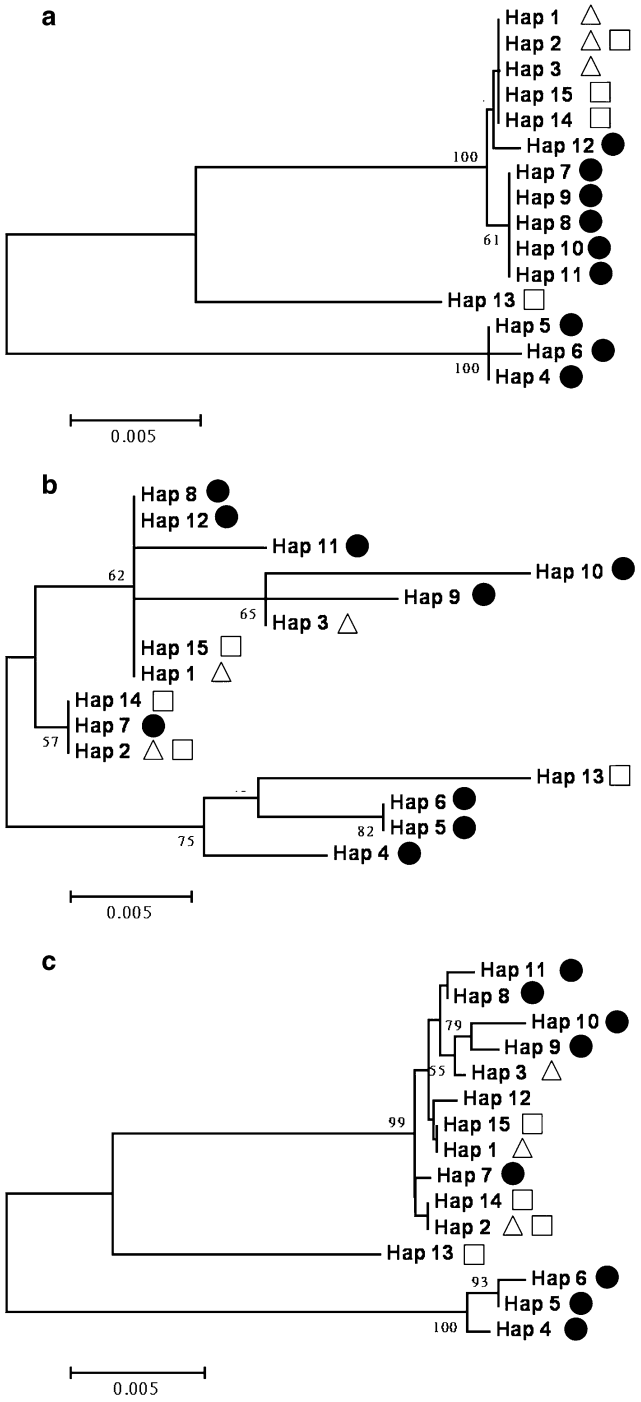

Fig. 3 Unrooted tree of *Ceriops tagal* from the Neighbor Joining method. (**a**) NJ tree constructed based on *atpB-rbcL* spacer of cpDNA; (**b**) NJ tree constructed based on *trnL-trnF* spacer of cpDNA; (**c**) NJ tree constructed based on *atpB-rbcL* + *trnL-trnF* spacers of cpDNA. Numbers on the branches are the bootstrap values of 1,000 replicates. ● are haplotypes distributed around the coasts of the Ocean of India; △ are haplotypes distributed around the east Malay Peninsula; □ are haplotypes in the Borneo. Scale bars indicate the inferred frequency of substitutions per nucleotide site

haplotypes in the Indian Ocean populations were all derived from those of the South China Sea (Fig. 4).

According to the nucleotide diversity estimated from every population (Table 2) and the genetic differentiation (G_{ST}, not shown), the genetic relationships of populations could be depicted. By the pairwise comparison of every population, there were clear relationships between the Borneo populations and eastern Malay Peninsula populations, than between the South China Sea and Indian Ocean populations (Fig. 6). In the UPGMA results, the Borneo and eastern Malay Peninsula populations had shorter genetic distances than did those of the western Malay Peninsula, while the geographic distance between eastern and western Malay Peninsula is shorter than the distance between the Malay Peninsula and Borneo.

Discussion

Genetic variation of *C. tagal* in Southeast Asia

Determining the population genetic variation is basic for exploring phylogeography. Many genetic markers have been used in different mangrove population research (Schwarzbach and Ricklefs 2001). Ge and Sun (2001) used the ISSR method to detect the variability in *C. tagal* from Thailand and Hainan Island (South China) and obtained a result of low genetic variation (with a mean genetic diversity, H_e, of 0.0084) including all populations. Although Ge and Sun (2001) concluded that a series of genetic bottlenecks during glacial epochs caused the low variation, Huang et al. (1999) had found a relatively high genetic variation in both east and west Malay Peninsula using isozyme analyses. In our current finding, cpDNA also revealed high haplotype diversity (Hd = 0.549), and most different haplotypes (12 of 15 haplotypes) were detected on the Malay Peninsula, concurring with Huang et al.'s conclusion that the Malay Peninsula is the center of genetic diversity of *C. tagal* in Southeast Asia.

Many genetic studies on mangrove species, such as *Avicennia marina* (Kado et al. 2004), *Kandelia candel* (Chiang et al. 2001; Kado et al. 2004), *Lumnitzera racemosa* (Kado et al. 2004), *C. decandra* (Tan et al. 2005), and *C. tagal* (Ge and Sun 2001) displayed limited genetic variability within local areas. In the eastern Malay Peninsula populations (SW, PT, and ST), relatively few haplotypes of cpDNA of *C. tagal* were detected. Restricted ocean currents may be a cause of speciation of mangrove species, such as the genus *Kandelia* (see Chiang et al. 2001; Sheue et al. 2003). The restricted ocean current in the Gulf of Thailand

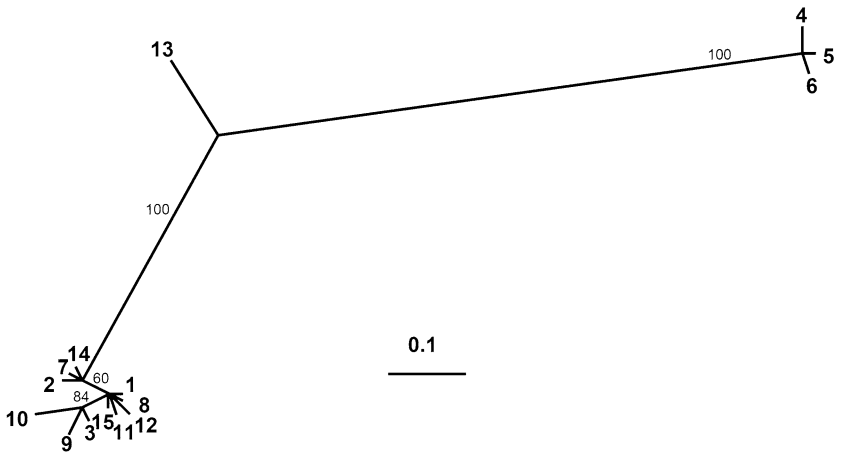

Fig. 4 Unrooted tree of *Ceriops tagal* from the Bayesian inference. Numbers on the tips of the branches are haplotype numbers, and those on the branches are the likelihood values by the MCMC estimation. The scale bar indicates the inferred frequency of substitutions per nucleotide site

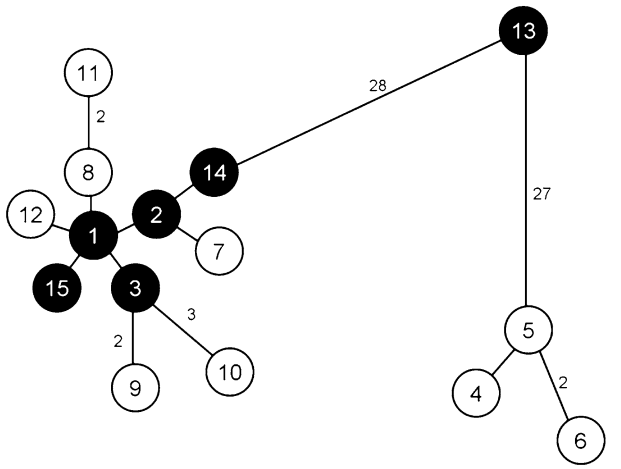

Fig. 5 Minimum spanning network of *Ceriops tagal*. The numbers in circles are haplotype numbers, and the numbers near the branches are the possible changes between haplotypes. Branches without the numbers indicate only one possible change between each other. The black circles indicate the South China Sea haplotypes and white circles are Indian Ocean haplotypes

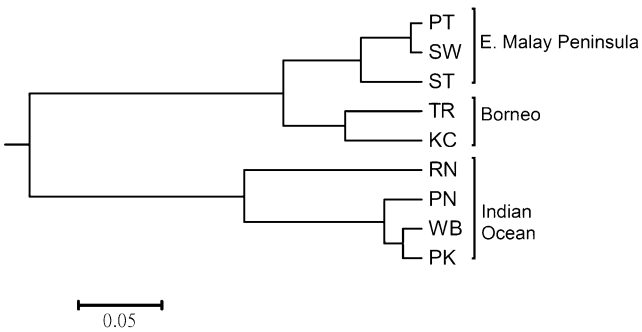

Fig. 6 UPGMA dendrogram of *Ceriops tagal* based on the G_{ST} of the *atpB-rbcL* + *trnL-trnF* spacers of cpDNA. The scale bar indicates the G_{ST} differences

may be the cause of limited propagule dispersal creating the low genetic variation on the eastern Malay Peninsula. The low dispersal ability of the propagules of *C. tagal*(McGuinness 1997; Clarke et al. 2001) could explain why so many haplotypes were endemic (Hap 4, 5, and 6 in RN, Hap 9 and 10 in PN, and Hap 11 and 12 in PK) on the Malay Peninsula. An endemic situation also occurred in Sabah, Borneo (Hap 13 and 15 in TR) (Fig. 1).

In the *atpB-rbcL* and *trnL-trnF* spacers of cpDNA, many differences were caused by long indel fragments, especially in Hap 4, 5, 6, and 13. In the analysis of NJ, BI, and the minimum spanning network, the clade containing Hap 4, 5, and 6 was always linked to Hap 13. However, only one indel fragment matched each other among all the gaps, and population RN (Hap 4, 5, and 6) is far from TR (Hap 13) in geographic distance (Figs. 3, 4 and 5). We have no evidence yet to explain the relatively close relationship of populations RN and TR. Some animals, such as monkeys, might take the propagules as food, but those animals seem to be incapable of long dispersal from Borneo to Malay Peninsula; on the other hand, the impaired propagules are unlikely to survive the distant journey (McGuinness 1997). We can only propose that the combination of the clades containing Hap 4, 5, and 6 and Hap 13 was possibly due to long-branch attraction (Felsenstein 1978).

The *trnL-trnF* spacer and *atpB-rbcL* spacer revealed different grouping patterns in the major clades might be a result of different rates of lineage sorting. We estimated the degrees of substitution rate (transition/transvertion rate) for both fragments, and found that none of them had reached saturation (data not shown). Thus, we can not exclude any fragments for analyzing purposes, and the following information of these fragments should be considered: the *atpB-rbcL* spacer has revealed a clearly geographic distribution pattern, and

the long term lineages sorting routs might be speculated by the *trnL-trnF* spacer.

Phylogeography of *C. tagal* in Southeast Asia

From the results of the network analysis (Fig. 4), Hap 1 and 2 may be the primitive genotypes in the South China Sea populations, and Hap 7 and 8 may be the primitive ones in the Indian Ocean populations. The geographical haplotype distribution reveals significant discrimination between eastern and western Malay Peninsula populations (Fig. 1). These results are consistent with the ISSR results of *C. decandra* (Tan et al. 2005) and *C. tagal* (Ge and Sun 2001). The land barrier of the Malay Peninsula caused *C. tagal* to differentiate at the genetic level. This is similar to how the Central American isthmus separates *Avicennia germinans* into West Atlantic Ocean populations and East Pacific Ocean populations (Dodd and Rafii 2002). A similar result of a land barrier was also observed in *A. marina* sensu lato in Indo-West Pacific populations (Duke et al. 1998). Duke et al. (1998) concluded that a land barrier between New Guinea and Australia during the last ice age had divided *A. marina* var. *eucalyptifolia* in the east and *A. marina* var. *marina* in the west according to isozyme markers. Even from the Paleocene to the recent period, the continued continental drift has progressively increased the efficiency of the land barrier (Plaziat et al. 2001).

About 17,000 years ago, the falling sea levels and the emergence of Sunda Land (Fig. 7) throughout the late Quaternary completely separated the Pacific and Indian Oceans (Voris 2000). Borneo and the Thai-Malay Peninsula were connected to each other because of the emergence of the Sunda Shelf. Mangroves formerly distributed around the Sunda Land coastline and the present-day mangrove populations around the Thai-Malay Peninsula and the northwestern Bornean coastline might have dispersed via the Sunda river system (Fig. 7). The genetic similarity among today's South China Sea populations might have been due to the coalescence of populations along the emergent coastline associated with the east Sunda River system. The east Borneo population (TR) had the most-different haplotype composition among the South China Sea populations. The former rivers of Sabah did not connect with nor were they adjacent to the other part of the east Sunda Land river system (see Fig. 7) (Voris 2000).

The dog-faced water snake, *Cerberus rnchops*, lives in the intertidal mud flats and mangrove forests, and has a similar biogeographic history with mangrove species. It is also differentiated between the Indian Ocean and South China Sea and has a similar phylogeographic story with *C. tagal* of sea-level changes and surface currents influencing the present-day genotype distribution (Alfaro et al. 2004). The isolation by the Sunda Land is also reflected in the genetic differentiation of the Kuda seahorse, *Hippocampus kuda*, in the Indian and Pacific Oceans (Lourie et al. 2005). The genetic differentiation of *C. tagal*, as well as those of other organisms in the Indo-West Pacific, reflects the past geographic history, especially sea level fluctuations during the Pleistocene. Like the restricted ocean current driving speciation of *Kandelia* in the East China Sea and South China Sea (Chiang et al. 2001; Sheue et al. 2003), our molecular data may indicate species diversification of *C. tagal* by vicariance due to the land barrier of the Malay Peninsula.

The haplotypes which located in the center of the networks are considered as the ancestral (original) states in the nested clade analysis (Templeton 2001). The minimum spanning network indicated that the Indian Ocean haplotypes were all derived from South China Sea populations (Fig. 5). Plaziat et al. (2001) drew possible dispersal routes of mangroves since 40 Mya: the fossil record displayed that *Ceriops* existed on European coasts in the early Eocene, then dispersed to East Asia in the middle Miocene (~16 Mya), finally to its present habitats. Plaziat et al. (2001) speculated the Paleo-African continent prevented the mangroves from dispersing from the Atlantic to the Indian Ocean. Instead, they might have dispersed through the Isthmus of Panama (North and South America did not adjoin each other in the Miocene) and crossed the Pacific Ocean to Asia and the Indian Ocean. This speculation suggests that some mangrove species expanded from Southeast Asia to South Asia instead of the reverse. Then the ocean currents influenced the recent mangrove dispersal (see Chiang et al. 2001). The clockwise surface current in the Bay of Bengal (Couper 1983) describes a possible dispersal route of *C. tagal* in this water body (Fig. 7). However, more-detailed sampling around the coasts of the Bay of Bengal and the Indian Ocean is needed to completely and accurately substantiate such phylogeographic speculations.

Conservation and management perspectives

In Southeast Asia, such as Thailand, the mangrove forests are one of the most abundant natural resources. Large scale logging in Southeast Asia rapidly consumes the natural environmental resources up to a rate of 1% of the mangrove forest per year (Robertson and Alongi

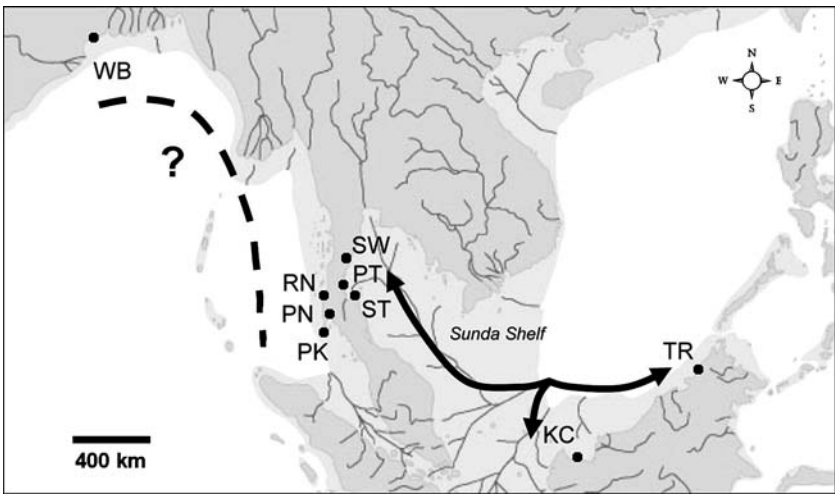

Fig. 7 Possible dispersal routes of *Ceriops tagal* in the South China Sea and Indian Ocean. The light gray area illustrates the maximum sea level drop during the last 17,000 years (Voris, 2000). The primary drowned river systems and the coastlines on the Sunda Shelf illustrate the possible dispersal routes of *C. tagal* in the South China Sea

1992). In Thailand, actively protect and rescue the environments and the forests via 41 Mangrove Management and Developmental Stations along the coasts of the gulf of Thailand and Andaman Sea. The afforestation is certainly a very effective restoring method; yet afforesting arbitrarily between stations results in the acceleration of the gene flow and genetic mixing between the populations. The afforestation approach, therefore, may decrease the genetic variability of the mangrove populations, despite the growth of mangrove-occupied areas, as well the increase in the habitats of wildlife. The population genetic structure greatly impacts the long-term evolution of species. Genetic mixing of mangroves introduced by human beings interferes with the natural function of the evolution. Our results indicate that the evolutionary history was the mainly cause of population differentiation. The detailed phylogeographic study of *C. tagal* can provide a list of suggested MUs for restoration purposes, thus prevent arbitrarily forestation. For example, the east and west coasts *C. tagal* of Malay Peninsula each contains unique and completely different genetic composition, which should not be mixed to each other by artificial restoration. Populations that have the significant distinct haplotypes need to be carefully protected, rather than being introduced to foreign seedlings. Thus, the donor propagules should be carefully selected according to their future neighboring population, in order to preserve the existing genetic population structure. We suggest that the authority responsible for the nature restoration should consider genetic population structure when planning reforestation.

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References

Alfaro ME, Karn DR, Voris HK, Abernathy E, Sellins SL (2004) Phylogeny of *Cerberus* (Serpentes: Homalopsinae) and phylogeography of *Cerberus rynchops*: diversification of a coastal marine snake in Southeast Asia. J Biogeogr 31:1277–1292

Chiang TY, Chiang YC, Chen YJ, Chou CH, Havanond S, Hong TN, Huang S (2001) Phylogeography of *Kandelia candel* in East Asiatic mangroves based on nucleotide variation of chloroplast and mitochondrial DNAs. Mol Ecol 10:2697–2710

Clarke PJ, Kerrigan RA, Westphal CJ (2001) Dispersal potential and early growth in 14 tropical mangroves: do early life history traits correlate with patterns of adult distribution? J Ecol 89:648–659

Couper A (1983) The times atlas of the ocean. Van Norstrand Reinhold Co., New York

Dodd RS, Rafii ZA (2002) Evolutionary of genetics of mangroves: continental drift to recent climate change. Trends Ecol Evol 16:80–86

Duke NC (1992) Mangrove floristics and biogeography. In: Robertson A, Alongi D (eds) Tropical mangrove ecosystem. American Geographical Union, Washington DC, pp 63–100

Duke NC (1995) Genetic diversity, distributional barriers and rafting continents – more thoughts on the evolution of mangroves. Hydrobiologia 295:167–181

Duke NC, Benzie JAH, Goodall JA, Ballment ER (1998) Genetic structure and evolution of species in the mangrove genus *Avicennia* (Avicenniaceae) in the Indo-West Pacific. Evolution 52(6):1612–1626

Duke NC, Lo EYY, Sun M (2002) Global distribution and genetic discontinuities of mangroves-emerging pattern in the evolution of *Rhizophora*. Trees 16:65–79

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. Genetics 136:343–359

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. Syst Zool 27:401–410

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752

Ge X-J, Sun M (2001) Population genetic structure of *Ceriops tagal* (Rhizophoraceae) in Thailand and China. Wetlands Ecol Manage 9:203–209

Huang S, Chen YJ, Havanond S (1999) Patterns of genetic variation of *Ceriops tagal* in Ranong and Eastern Malay Peninsula. Paper presented at Ecotone VIII. May 23–29, 1999, Ranong, Thailand

Kado T, Fujimoto A, Giang LH, Tuan M, Hong PN, Harada K, Tachida H (2004) Genetic structures of natural populations of three mangrove species, *Avicennia marina, Kandelia candel* and *Lumnitzera racemosa*, in Vietnam revealed by maturase sequences of plastid DNA. Plant Species Biol 19:91–99

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Briefings Bioinformatics 5:150–163

Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol Ecol 14:1073–1094

Maguire TL, Saenger P, Baverstock P, Henry R (2000) Microsatellite analysis of genetic structure in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae). Mol Ecol 9:1853–1862

McGuinness KA (1997) Dispersal, establishment and survival of *Ceriops tagal* propagules in a north Australian mangrove forest. Oecologia 109:80–87

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. Mol Ecol 3:401–411

Nei M (1987) Molecular evolutionary genetics. Columbia Univ. Press, New York

Newton AC, Allnutt TR, Gillies ACM, Lowe AJ, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol Evol 14:140–145

Plaziat J-C, Cavagnetto C, Koeniguer J-C, Baltzer F (2001) History and biogeography of the mangrove ecosystem, based on a critical reassessment of the paleontological record. Wetlands Ecol Manage 9:161–179

Robertson AI, Alongi DM (1992) Tropical mangrove ecosystems (coastal and estuarine studies; vol. 41), American Geophysical Union

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rozas J, Sanchez-Delbarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. Trends Ecol Evol 1:9–10

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. Mol Ecol 7:465–474

Schwarzbach AE, Ricklefs RE (2001) The use of molecular data in mangrove plant research. Wetlands Ecol Manage 9:195–201

Sheue CR, Liu HY, Yong JWH (2003) *Kandelia obovata* (Rhizophoraceae), a new mangrove species from Eastern Asia. Taxon 52:287–294

Shi S, Zhong Y, Huang Y, Du Y, Qiu X, Chang H (2002) Phylogenetic relationships of the Rhizophoraceae in China based on sequences of the chloroplast gene *matK* and the internal transcribed spacer regions if nuclear ribosomal DNA and combined data set. Biochem Syst Ecol 30:309–319

Sun M, Wong KC, Lee JSY (1998) Reproductive biology and population genetic structure of *Kandelia candel* (Rhizophoraceae), a viviparous mangrove species. Am J Bot 85(11):1631–1637

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Tan F, Huang Y, Ge X, Su G, Ni X, Shi S (2005) Population genetic structure and conservation implications of *Ceriops decandra* in Malay Peninsula and North Australia. Aquat Bot 81:175–188

Templeton AR (2001) Using phylogeographic analyses of gene trees to test species status and processes. Mol Ecol 10:779–791

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tomlinson PB (1986) The botany of mangroves. Cambridge of University Press, Cambridge

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27:1153–1167

Zhong Y, Shi S, Tang X, Huang Y, Tan F, Zhang X (2000) Testing relative evolutionary rates and estimating divergence time among six genera of Rhizophoraceae using cpDNA and nrDNA sequences. Chinese Sci Bull 45:1011–1015