Population subdivision promoted by a sea-level-change-driven bottleneck: a glimpse from the evolutionary history of the mangrove tree Aegiceras corniculatum

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Abstract

Historic climate changes had always driven geographical populations of coastal plants to contract and recover dynamically, even die out completely. Species suffering from such bottlenecks usually lose intraspecific genetic diversity, but how do these events influence population subdivision patterns of coastal plants? We investigated this question in the typical coastal plant: mangrove species Aegiceras corniculatum. Inhabiting the intertidal zone of the tropical and subtropical coast of the Indo-West Pacific oceans, its populations are deemed to be greatly shaped by historic sea-level fluctuations. Using dual methods of Sanger and Illumina Solexa sequencing, we found that the 18 sampled populations were structured into two groups, namely, the "Indo-Malayan" group, comprising three subgroups (the northern South China Sea, Gulf of Bengal, and Bali), and the "Pan-Australasia" group, comprising the subgroups of the southern South China Sea and Australasia. Based on simulations using the approximate Bayesian computation method, we inferred that the southern South China Sea subgroup, which penetrates the interior of the "Indo-Malayan" group, originated from the Australasia subgroup, accompanied by a severe bottleneck event, with a spot of gene flow from both the Australasia and "Indo-Malayan" groups. Geographical barriers such as the Sundaland underlie the genetic break between Indian and Pacific Oceans, but the discontinuity between southern and northern South China Sea was originated from genetic drift in the bottleneck event. Hence, we revealed a case evidencing that the bottleneck event promoted population subdivision. This conclusion may be applicable in other taxa beyond coastal plants.

Introduction

To what range a species can distribute and how its intraspecific genetic variations are structured are fundamentally determined by geographical and ecological factors (Melville & Burchett, 2002). Various forms of geographical barriers, on the one hand, constrain the margins of a species' distribution ranges and, on the other hand, cause abrupt discontinuities within the range (Hartl & Clark, 1997). To the coastal plants that disperse propagules by seawater, the geographical barriers are usually landmasses, open oceans (Thornhill, Mahon, Norenburg, & Halanych, 2008), and ocean currents. Moreover, limitations of dispersal ability may further shape their populations, following a pattern called isolation by distance (IBD), which assumes that the level of differentiation is positively correlated with geographic distance.

The geographic landscape and ecological landscape are not persistent throughout history, making the populations of organisms highly dynamic. Geological actions had changed the geographical landscape gradually in a macroevolutionary timescale, such as the uplift of the Qinghai-Tibetan Plateau (Ramstein, Fluteau, Besse,

& Joussaume, 1997; Zhisheng, Kutzbach, Prell, & Porter, 2001) and the closure of the Central America isthmus (Lessios, 2008). In a smaller timescale, the historic climate changes, e.g. the Quaternary glaciers, had reshaped the Earth continuously. For coastal plants, the historic sea-level changes due to climate changes had caused geographic barriers to emerge and vanish repeatedly. The emergence of a barrier may subdivide populations while the vanish of a barrier may reunite diverging populations (Ge et al., 2015). Hence, the population structure within a species is an integrated output of the forces dividing populations and those mixing populations.

Other than isolating and connecting populations, historic geographical and climatic changes also shaped the demographic size of populations. Typically, the populations of a species contract to refugia or even become extinct when geographic or ecological conditions are too hostile to survive (Foufopoulos, Kilpatrick, & Ives, 2011; Hallam & Wignall, 1999; Jackson, Winston, & Coates, 1985; Ozawa, 2010; Paulay, 1990), and recover and expand when the conditions are ameliorated. During these processes of "shrinkage-expansion" (i.e., bottleneck), even when the species survive, a reduction in intraspecific diversity is common (Haanes, Røed, Flagstad, & Rosef, 2010; Mamuris, Stoumboudi, Stamatis, Barbieri, & Moutou, 2005; Moum & Árnason, 2001; Tsuchida, Kudô, & Ishiguro, 2014). In addition to reducing intraspecific diversity, how would such a bottleneck process influence the population structure within a species? During the "shrinkage-expansion" process, the refugial population usually sampled only a small subset of the total ancestral polymorphisms, and the small population is more likely to lose inherent polymorphisms and fix new mutations due to intensified genetic drift and relaxed purifying selection. Moreover, the contracted population is usually less likely to exchange genes with other populations due to enhanced isolation. Hence, we hypothesized the bottleneck process is highly potential in generating novel genetic structures within species.

Several previous studies have referred to this issue indirectly. In a coral reef fish, the strong nonequilibrium genetic structure was shown to be generated by genetic bottlenecks/founder effects associated with population reduction/extinctions and asymmetric migration or recolonization (Bay, Caley, & Crozier, 2008). Repeated bottleneck events during colonization of the parasite *Geomydoecus aurie* have been shown to impart genetic structure of a population due to allele surfing (Demastes, Hafner, Hafner, Light, & Spradling, 2019). The fine-scale genetic structure of *Rhizophora racemosa* in the Cameroon estuary complex was attributed to contemporary processes such as restricted propagule dispersal, bottleneck events, and recolonization by founders from ancient local refugia (Ngeve, Van der Stocken, Menemenlis, Koedam, & Triest, 2017). Despite the awareness that bottleneck may generate genetic structure, the hypothesis needs to be comprehensively and explicitly addressed.

The model with which to test this hypothesis should live in a highly dynamic habitat and be sensitive to habitat change. Mangrove plants, a group of typical coastal plants, are therefore an ideal model. Mangrove plants are distributed linearly along coasts and strictly inhabit tropical and subtropical intertidal zones. In the Quaternary cycles of glacial and interglacial conditions, climate changes drove the sea level to change in cycles (Miller et al., 2005; Zachos, Pagani, Sloan, Thomas, & Billups, 2001). The coastlines shifted following sea-level rise and drop, and mangrove forests were forced to advance and retreat repeatedly (Woodroffe & Grindrod, 1991). Hence, we tested the above hypothesis in mangrove plants, but the underlying reasonability may be general in other taxa.

Several studies had determined the genetic structure of different mangrove species, with periodic geographical barriers frequently identified in places such as the Malay Peninsula and the Wallacea region (W. Guo et al., 2018; Z. Guo et al., 2016; J. Li et al., 2016; Urashi, Teshima, Minobe, Koizumi, & Inomata, 2013; Y. Yang et al., 2016, 2017), in addition to a set of permanent barriers (Duke, 2017). As the historic sea-level changes eroded barriers periodically, populations of mangrove species were isolated or connected by intermittent gene flow, which was previously employed to illustrate the observed population structure (divergence and admixture) of many coastal species (Banerjee et al., 2020, 2021; Z. Guo, Guo, et al., 2018; Z. Guo et al., 2016; Westberg & Kadereit, 2009). Such intermittent gene flow was demonstrated to have promoted the speciation of mangrove species via a "mixing-isolation-mixing" mechanism (Z. He et al., 2019). However, it's unclear how demographic size changes driven by sea-level changes have shaped population structure,

although some studies have also reported that bottleneck events were involved in some mangrove species' demographic histories and caused population diversity reductions (W. Guo et al., 2020, 2018; Zhou et al., 2010). It's intriguing to test the hypothesis that bottleneck events could promote population subdivision. We performed such a study in the typical mangrove plant *Aegiceras corniculatum* , which is always a frontier species in mangrove forests.

Aegiceras corniculatum is distributed widely from India across Southeast Asia and South China to Australia and west Pacific islands (Duke, 2014), likely facilitated by its long-distance dispersal via buoyant propagules. Ge and Sun et al. had used inter-simple sequence repeat markers to reveal between-population differentiation in the populations of *A. corniculatum* in China (Ge & Sun, 1999). Deng et al. had used amplified fragment length polymorphism markers to reveal genetic divergences among populations in China, Malay Peninsula, and Sri Lanka (Deng et al., 2009). However, the genetic pattern of its whole distribution range remains unaddressed. We sampled 18 populations, covering the distribution range of *A. corniculatum* , and used both Sanger and Illumina Solexa sequencing to obtain single nucleotide polymorphism (SNP) markers, with which we could ascertain the population structure of *A. corniculatum* across its distribution range. Due to the dominant role of Sundaland and Wallacea barrier in coastal plants in the IWP, we expected to see the populations of *A. corniculatum*from the Indian Ocean, Southeast Asia (including South China), and Australasia is exclusively grouped. Moreover, we expected the historic sea-level changes had shaped demographic size changes of *A. corniculatum* populations, particularly, bottleneck event in history is expectable. Particular interest was devoted to whether bottleneck events had played a role in generating additional population structure not attributed to geographic barriers. The findings will deepen our understanding of population evolution in dynamic geographic and climate changes and guide efforts to conserve genetic diversity below the species level.

METHODS

Plant materials and sequencing

A total of 631 *A. corniculatum* individuals from 18 sites in the Indo-West Pacific region were collected (Figure 1a, Table 1). The sampled individuals were at least 5 m apart, and one leaf was collected from each individual. DNA of each individual was extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987).

Referencing the transcriptome of an individual from Hainan, we newly designed 93 pairs of primers anchored at the nuclear genome of *A. corniculatum* , with each pair of primers anchoring at exons but spanning at least one intron. Six genes (A189, A245, A383, A414, A440, and C058) were amplified in 10 to 37 individuals from the 18 populations (423 in total) (Figure 1, Table 1). The amplicons were sequenced using the Sanger method on an ABI 3730 platform at Huada Genomic Institute (BGI) (Shenzhen). The PCR procedure for amplification was as follows: 4 min at 94°C; 30 cycles of 15 s at 94°C, 15 s of annealing at 53°C, and extension at 72°C for 2 min; and a final 10 min extension at 72°C. The reactions were held at 10 before the PCR products were subjected to electrophoresis on 1% agarose gels.

To corroborate the pattern uncovered by the six Sanger-sequenced genes, we sequenced all 93 genes against 11 populations (491 individuals in total) on an Illumina GA platform at Huada Genomic Institute (BGI) (Shenzhen). For each population, we pooled equal amounts of DNA from all the individuals of the population. For each of the 93 genes, we amplified the pooled DNA using the same procedure described above, and the purified PCR products of all 93 nuclear loci from one population were further pooled in equal quantities to reach a total of 10 μg for sequencing.

Sequence analysis and genetic diversity estimation

The sequences of 18 populations obtained from Sanger sequencing were aligned and edited in SeqMan 7.1.0. For each population, the nucleotide diversity (π) and DNA polymorphism (Watterson's ϑ) were calculated using DnaSP 5.10 (Librado & Rozas, 2009).

We obtained the reference sequences of the 93 genes by sequencing one*A. corniculatum* individual using the

Sanger method (see the supplementary file of He et al., 2019). The reference sequences ranged in length from 203 to 2422 bp. The short reads produced from Illumina sequencing were mapped to reference sequences using MAQ 0.7.1 (H. Li, Ruan, & Durbin, 2008) with the parameters set such that the mutation rate between the reference and read was set to 0.002, the threshold of mismatch base quality sum was 200, and the minimum mapping quality of the reads was 30. To exclude false-positive mismatches, we counted the mismatch rate for each site across the read and the mismatch rate for each base quality. We trimmed the first and last 10 bases of each read and filtered bases with a quality score of less than 20. Single nucleotide polymorphisms (SNPs) were also identified using MAQ 0.7.1 (H. Li et al., 2008). To avoid introducing bias from sequencing errors, we discarded the sites with insufficient site coverage (<100 reads) and those with minor allele frequency less than 1/2N (N is the number of individuals) in each population (Z. He et al., 2013). The allele frequencies for each SNP site in a population were obtained by counting the depth of each allele.

For the Illumina data, we estimated the nucleotide polymorphism (Watterson's ϑ) of each gene using the method of He et al. (Z. He et al., 2013). The nucleotide diversity (π) of each gene was also estimated according to Nei's formula (Nei, 1987) with an in-house script. To estimate absolute genetic divergence between populations, we computed pairwise D_{XY} following the formula derived by Nei (Nei & Li, 1979). Pairwise D_{XY} values were summed over all SNPs, and the sum was normalized by effective sequence length. For each pair of populations, the effective sequence length was defined by sites without missing data in either population. We also estimated Wright's F statistics (F_{ST}) (Wright, 1950) with these data.

Inferring population structure

To identify the genetic structure of *A. corniculatum* , 423 individuals from 18 populations with Sanger sequences were assigned into a putative number of clusters using a Bayesian clustering approach with STRUCTURE 2.3.4 (Falush, Stephens, & Pritchard, 2003; Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard, Stephens, & Donnelly, 2000). The program identified the K genetic clusters of origin of the sampled individuals and assigned the individuals simultaneously to the genetic clusters by calculating the posterior probability. The maximum K was set to 12, and for each K, 10 replicates were conducted. Each run consisted of 1×10^6 Markov chain Monte Carlo (MCMC) iterations with a burn-in of 2×10^5 under an assumed model of admixture and correlated allele frequencies. The most likely K was determined by the delta K statistic using STRUCTURE HARVESTER (Evanno, Regnaut, & Goudet, 2005). The population structure results are shown graphically by DISTRUCT 1.1(Rosenberg, 2004), and each individual is a line segment partitioned into K coloured components, which represent the individual's estimated membership coefficients in the K clusters.

We also performed principal component analysis (PCA) on the SNP frequency matrix (summarizing the frequency of each SNP in each Illumina-sequenced population) using the "princomp" function in R (Venables & Ripley, 2013) to test whether the SNP frequencies differed among populations. Using the "pegas" R package, the analysis of molecular variance (AMOVA) was performed to characterize the hierarchical assignment of variance components at levels of population and cluster of populations. We performed this analysis for each of the six Sanger-sequenced genes.

The revealed genetic structure was further checked gene by gene by constructing a haplotype network for each gene and mapping the haplotypes geographically. Haplotypes of six nuclear genes across the 18 populations were inferred using DnaSP 5.10 (Librado & Rozas, 2009), and the networks were constructed by an expectation-maximization algorithm with *A. floridum* as the outgroup. The networks were visualized using NETWORK 5.0 (*http://www.fluxus-engineering.com/*) (Bandelt, Forster, & Röhl, 1999) and plotted on a map using GenGIS (Parks et al., 2009).

The 93 genes sequenced by the Illumina platform were also used to infer haplotypes using the method developed by (Z. He et al., 2019). He et al. validated the accuracy of this method to infer haplotypes by sequencing individuals using the Sanger method. The details associated with using this method have been described in a previous publication (Wang et al., 2021). We obtained 392 gene segments and 84 gene segments

longer than 300 bp, and a haplotype network was also constructed for each segment longer than 300 bp using NETWORK 5.0 (*http://www.fluxus-engineering.com/*) (Bandelt et al., 1999).

Inferring geographical barriers

To identify the biogeographical boundaries that exhibit the largest genetic discontinuities between population pairs, we used the F_{ST} matrix as the distance matrix to calculate the Monmonier maximum difference by BARRIER 2.2 (Manni, Guérard, & Heyer, 2004). To ensure robustness, we randomly selected 30 genes from the 93 genes to accumulate one F_{ST}matrix. We repeated this process 100 times and obtained 100F_{ST} matrices. The robustness of each barrier was assessed by bootstrapping over the 100 matrices of genetic differentiation.

Mantel tests of F_{ST} against geographic distance were performed to test the isolation by distance model. We used the spheric distance of pairwise sampling sites to approximate geographic distance. The test was conducted with 1000 permutations and at two levels: all populations, and populations of a genetic group.

Demographic history simulation

The populations were distinctly structured, basing on the abovementioned analyses. The populations in the southern South China Sea (Lineage 1, including Chaiya and Kuching) are closely related to those in Australasia (Lineage 2, including U-Daintree, Darwin, and Sorong) instead of those geographically surrounding them (Lineage 3, including Sanya, Wenchang, Yalong, La-un, Ngao, and Bali). To examine how the populations in the southern South China Sea originated, we built 12 models and used the approximate Bayesian computation (ABC) method to choose the optimal model. The 12 models were first different in population topology: (1) Lineage 2 diverged with Lineage 3 first and then Lineage 1 diverged from Lineage 3 (Models 1-4); (2) Lineage 2 diverged with Lineage 3 first and then Lineage 1 diverged from Lineage 2 (Models 5-8); and (3) Lineage 2 diverged with Lineage 3 first and then Lineage 1 derived from an admixture of Lineage 2 and Lineage 3 (Models 9-12). Within each topology, the four models differed in population reduction, population bottleneck, population reduction with gene flow, and population bottleneck with gene flow.

Simulated sequences under these models were produced by ms software (Hudson, 2002). To reduce the complexity of the parameters, we derived population size parameters from a single N_0. N_0 was randomly picked from the prior distribution and assigned to Lineage 1 as the baseline. The population size of the other two lineages (N_x) is equal to ϑ_x/ϑ_0, where ϑ_x and ϑ_0 are the observed ϑ of the current and baseline lineages, respectively. We performed 1×10^5 simulations using the ms program for each model (Hudson, 2002). Eighty loci of 1000 base pairs were simulated in each run with the mutation rate set at $4.06\text{x}10^{-8}$ per generation per bp. The mutation rate was estimated from phylogenomic comparisons to closely related species (Z. He et al., 2019). The sample size of each group was set equal to the corresponding cluster pooling in multiple populations. Uniform prior distributions were set for the demographic parameters, with corresponding parameters in different models having an identical prior range (Table S1 in supplementary file).

For each simulation, we calculated 18 summary statistics, including Watterson's estimator (ϑ), nucleotide polymorphism (π), segregating sites (S), and Tajima's D within each group andD_{XY} and F_{ST} for each pair of groups. To perform model selection, we calculated Euclidean distances by comparing the summary statistics of the simulated and observed sequences. We set the tolerance as 0.05 to retain the simulated data, and then the approximate Bayesian computation schema (Beaumont, Zhang, & Balding, 2002) was used to estimate the Bayesian posterior probabilities of each mode using the "abc" package in R (Csilléry, François, & Blum, 2012). The "postpr" function and "neuralnet" option were used. With the optimal model selected, the "neuralnet" method in the "abc" R package was again used to estimate the posterior distribution of demographic parameters.

RESULTS

Genetic diversity of *A. corniculatum* at the population and species levels

Using next-generation sequencing techniques, we obtained 63 to 75 kb of DNA sequences covering 69 to 82 genes in the 11 populations (Table 2), ranging in length from 197 to 2301 bp. The average sequencing depth of the Sanya, Wenchang, and Yalong populations was more than 2200 x, whereas that of the other eight populations was more than 4400 x. By mapping short reads to reference sequences, we identified 91 to 761 segregating sites within each population (Table 2). The π and ϑ for each Illumina-sequenced gene indicated a high level of genetic variation at the species level but relatively low genetic diversity within populations and regions (Figure 1b&c). Extremely low π and ϑ were observed in the populations Chai-ya and Kuching from the southern South China Sea, significantly lower than those in the Gulf of Bengal (La-un and Ngao, P-value < 0.001, Wilcoxon test) and in Australasia (Sorong, Darwin, and U-Daintree, P$<$0.001, Wilcoxon test) (Figure 1, Table 2). The genetic diversity of Chai-ya and Kuching are also lower than those in Hainan Island, though not that significant (P-value=0.026 for π and P-value < 0.001 for ϑ, Wilcoxon test). The genetic diversity of populations without Illumina data was evaluated from the six nuclear genes sequenced by the Sanger method. Most of the populations from the northern South China Sea and Australasia have medium values of π and ϑ, and the two marginal populations in Siri Lanka composite a relatively low level of genetic variation (Figure 1, Table 2).

Population structure within the distribution range of *A. corniculatum*

The Sanger sequences of 423 individuals from 18 populations were used to identify the uppermost hierarchical level of the genetic clusters. The optimal K was estimated to be two according to Evanno's method (Figure S1). One cluster included the Bali, La-un, Ngao, Bangladesh, Pambala, Rekawa, Dongzhai, Danzhou, Sanya, Wenchang, and Yalong populations, which cover almost the whole Asian range, excepting the southern South China Sea. This cluster is defined as the "Indo-Malayan" group. The other cluster included the Kuching, Chai-ya, Sibu, U-Daintree, L-Daintree, Darwin, and Sorong populations, which cover the whole Australasia region and the southern South China Sea (Figure 2). We call the second cluster the "Pan-Australasia" group.

To test whether a substructure exists within each group defined above, the same STRUCTURE clustering was applied to each group. The optimal K in the "Indo-Malayan" group was estimated to be four according to Evanno's method (Figure S2). The populations around the Gulf of Bengal ("Gulf of Bengal" subgroup, including La-un, Ngao, Bangladesh, Pambala, and Rekawa) share a component, the single Bali population on Java Island ("Bali" subgroup) constitutes a component, and the remaining northern South China Sea populations ("n-SCS" subgroup, including Dongzhai, Wenchang, Danzhou, Sanya, and Yalong) consisted of two admixed components (Figure 2). Hence, the "n-SCS" populations are more plausibly considered as one subgroup. In the Pan-Australia group, two components (Figure S3) are distributed in the southern South China Sea ("s-SCS" subgroup, including Chai-ya, Kuching, and Sibu) and Australasia ("Australasia" subgroup, including U-Daintree, L-Daintree, Darwin, and Sorong) (Figure 2). Hence, the 18 populations were reasonably clustered into two groups and further clustered into five subgroups.

The clustering pattern revealed by the Sanger sequencing data was validated using Illumina data. The PCA clustering based on the SNP frequency matrix revealed an approximately consistent pattern: (1) the "s-SCS" subgroup (represented by Kuching and Chai-ya) and "Australasia" subgroup (represented by U-Daintree, Darwin, and Sorong) grouped in the upper right corner; (2) the "n-SCS" populations (represented by Sanya, Yalong, and Wenchang) were grouped in the lower-left corner; and (3) the populations of the "Gulf of Bengal" (represented by La-un and Ngao) were grouped between the "n-SCS" population cluster and the single population ("Bali"). Hence, the differentiation between the "Indo-Malayan" and "Pan-Australasia" groups is higher. Within the "Indo-Malayan" group, the "n-SCS" subgroup may be less different from the "Gulf of Bengal" subgroup than the "Bali" subgroup.

The F_{ST} and D_{XY} statistics provide a direct estimation of population differentiation and divergence. The F_{ST} values estimated from Sanger data agree well with the clustering pattern revealed above (Figure 2a). Both datasets show lower F_{ST} values between populations within each subgroup (ranging from 0.07˜ 0.39 estimated from Solexa data and 0.01 to 0.54 from Sanger data), while higher F_{ST} values were observed between populations from different subgroups (0.42˜0.53 from Solexa data and 0.37˜0.92 from Sanger data) (Figure 2a

and S4). We performed the AMOVA to determine the hierarchical percentages of variation, basing on the F_{ST} matrix of each of the six Sanger-sequenced genes. The majority of variation components (83.63˜97.20%) were attributed to "among subgroups" in all genes, excepting the gene A414 (40.13%) (Table S3). Consistently, little variation was attributed to "among populations within subgroup" or "within populations" (Table S3). TheD_{XY} statistics estimated from the six Sanger-sequenced genes showed a very clear divergence between the five subgroups but an obviously lower divergence between populations within each subgroup (Figure S5). Interestingly, the D_{XY}estimated from Solexa data did not show high divergence (0.78- 3.01) among the population pairs around the SCS (including populations in the "n-SCS" and "s-SCS", Figure S6).

Haplotype grouping and geographical distribution

The haplotype networks constructed from all six Sanger-sequenced genes robustly showed the major break between Asia and Australasia. Particularly, within each of the five defined subgroups, the populations compose the same cluster of closely related haplotypes in all six genes. However, it's observed in almost all genes that two or more subgroups shared the same cluster of haplotypes. In the A189 gene, the "Indo-Malayan", "s-SCS" and "Australasia" populations compose distinct haplotypes (Figure 3a). Within "Indo-Malayan", haplotypes in "Gulf of Bengal" are different from those in "n-SCS". In gene A414, the whole "Indo-Malayan" group shared haplotypes, with the two populations on the west coast of the Malay Peninsula (La-un and Ngao) having distinctive haplotypes. A cluster of haplotypes also occurred strongly in Chai-ya and to a lesser extent in other populations of the South China Sea (Figure 3b). In the A440 gene, the "Indo-Malayan" and "Pan-Australasia" populations show distinct haplotypes (Figure 3c). For the A245 gene, each of the "s-SCS", "n-SCS", "Gulf of Bengal" and "Australasia" subgroup show distinct haplotypes (Figure 3d). In the C058 gene, "s-SCS" share haplotypes with the "Bali" subgroup, while "Australasia" and "Indo-Malayan" (excepting Bali) show distinct haplotypes (Figure 3e). In the A383 gene, the "s-SCS", "Australasia" and "Bali" populations shared a cluster of haplotypes, while the "n-SCS" and "Gulf of Bengal" populations had divergent haplotypes (Figure 3f).

As the six Sanger-sequenced genes showed contrasting patterns, we constructed haplotypes from the Illumina sequences. Haplotypes were inferred based on the linkage information of short reads, with the 93 genes split into segments (see Methods). We retained the 84 segments with lengths longer than 300 bp for the following analyses (Table S2). Forty-three of the 84 segments showed no divergent haplotype groups, with their haplotype networks showing a loop- or star-like topology. The remaining 41 segments showed 2-5 divergent clusters. (1) Twelve segments split into three clusters, with one distributed in the "Indo-Malayan" populations, the second in "s-SCS" and the last in "Australasia" (Figure 4a); (2) eight segments split into two clusters, with one in Asia (including the "Indo-Malayan" and "s-SCS") and the other in "Australasia" (Figure 4b); (3) six segments split into two clusters: "Indo-Malayan" and "Pan-Australasia" (Figure 4c); (4) six segments fell into five divergent haplotype groups, with each of the five second-level group composites a cluster (Figure 4d); (5) five segments split into three clusters: "n-SCS and Gulf of Bengal", "s-SCS and Bali", and "Australasia" (Figure 4e); and finally, (6) four segments split into three clusters: "n-SCS", "s-SCS, Gulf of Bengal and Bali", and "Australasia" (Figure 4f).

Geographical barrier identification and test of isolation by distance

By calculating Monmonier's maximum difference using the program BARRIER, we inferred four strong geographic barriers. The robustness of each barrier was assessed by bootstrapping over 100 matrices ofF_{ST} , with each matrix calculated from 30 genes randomly sampled from the 93 sequenced genes. The first barrier, supported by the 99 F_{ST} matrix, aligns with the Malay Peninsula, isolating La-un and Nago from Chai-ya (Figure 5a). The second barrier, with the support of 85 matrices, lies in the Indonesian archipelago, isolated Bali from Kuching (Figure 5a). Both of the two barriers are part of the Sundaland barrier. The third barrier lies in the Wallacea region, supported with 93,75 and 75 matrices (Figure 5a). This barrier isolated the populations in Australasia from those in Southeast Asia. The fourth barrier, which is supported by 95, 95, and 59 matrices, lies between the northern South China Sea and southern South China Sea (Figure 5a).

The Mantel test revealed a significant correlation (r =0.518, P<0.01) between geographical distance and

genetic differentiation of Sanger F_{ST} estimations (Figure 5b). The same test using Solexa data also showed a significant correlation (r=0.485, P-value < 0.01, Figure S7). The Mantel tests within the "Pan-Australasia" group (r=0.560, P-value <0.01, Figure S8) and "Indo-Malayan" group (r=0.698, P-value <0.01, Figure S9) showed even more stronger correlations. This indicated isolation by distance had contributed to the population differentiation. Despite the significant r-value, the population structures revealed before were evidenced by that the population pairs within subgroups showed much smaller F_{ST} values than the IBD expectation while the population pairs of s-SCS vs. n-SCS or s-SCS vs. the Indian Ocean showed much largerF_{ST} (Figure 5b).

Bottleneck event revealed by simulations

We employed the approximate Bayesian computation (ABC) method to test 12 possible models (Figure 6). By simulations, model 8 acquired the highest posterior probability, which assumed that the "s-SCS" populations shared a common ancestor with "Australasia", then "s-SCS" diverged with "Australasia", accompanied by a severe bottleneck, and recently expanded to its current population size (Figure 6). Migrations between the "Indo-Malayan" and "s-SCS" or between "Australasia" and "s-SCS" have occurred in recent times. This model choice is robust, with a posterior probability larger than 0.6 in all three repeated computations (Table 3).

Under the optimal demographic history model, we estimated the posterior distribution of the demographic parameters. The "Indo-Malayan" lineage and the ancestor of the "Australasia" and "s-SCS" populations (the "Pan-Australasia" cluster) diverged approximately 2.7 million years ago (95% confidence interval, i.e. CI: 2.6˜3.0). Next, the "s-SCS" lineage diverged from the "Australasia" populations approximately 1.5 million years ago (95% CI, 1.2˜1.9). N_0 was estimated as 1916. Accordingly, the population size of the ancestral "s-SCS" group was estimated to have reduced from 10538 to 824 (5.5 N_0 to 0.43 N_0) in the bottleneck event. Since approximately 0.13 million years ago (95% CI 0.1˜0.2), theN_e of the "s-SCS" group has increased gradually to 1917 (95% CI, 1812˜1986). TheN_e values of the "Indo-Malayan" and "Australasia" groups were relatively constant, at 9580 and 10538, respectively. Gene flow was asymmetric, that the values are 0.258 per generation (95% CI, 0.118˜0.688) from the "Indo-Malayan" to the "s-SCS" populations and 0.007 (95% CI, 0.002˜0.021) in the reciprocal direction. Similarly, the gene flow was 0.403 (95% CI, 0.216˜0.775) per generation from the "Australasia" to the "s-SCS" populations, and the reciprocal flow was 0.005 (95% CI, 0˜0.075) per generation (Table 4).

DISSCUSSION

Geographical barriers played a fundamental role in shaping genetic structuring of coastal plant species

In addition to open oceans that are too wide for propagules to pass through, land barriers and ocean currents are commonly found to hinder the dispersal of coastal plant species that disperse propagules via seawater (Kadereit, Arafeh, Somogyi, & Westberg, 2005; H. Yang, Lu, Wu, & Zhang, 2012). Mangrove species, which are the most typical coastal plants, strictly inhabit the intertidal zones and disperse via buoyant propagules such as fruits, seeds, and hypocotyls. The propagules of some species can float at sea for months before reaching a new intertidal habitat (Tomlinson, 2016). However, the phylogeographical pattern of mangrove species had been fundamentally shaped by land barriers and ocean currents, such as the Central American Isthmus to *Rhizophora mangle* or *R. racemosa* (Takayama, Tamura, Tateishi, Webb, & Kajita, 2013) and the ocean currents at the northeastern extremity of South America to *R. mangle* , *Avicennia germinans* or*Avicennia schaueriana* (Mori, Zucchi, & Souza, 2015; Pil et al., 2011).

The Indo-West Pacific region, particularly Southeast Asia, is the hotspot of mangrove species diversity (Duke, 2017), thus most of the studies discussing phylogeography of mangrove species concentrated on this region. The Malay Peninsula (or the Sundaland when sea level was low) was identified to be the most dominant land barrier for mangrove species. Genetic discontinuity between the Indian ocean and Pacific Ocean sides of Sundaland has been observed in *R. apiculata* (Z. Guo et al., 2016), *R. mucronata* (Yan, Duke, & Sun, 2016), *Avicennia marina* (Wang et al., 2021), *Sonneratia alba* (Y. Yang et al., 2017), *S. caseolaris* (Y. Yang et

al., 2016), *Ceriops tagal* (Huang et al., 2012), *C. decandra* (Huang et al., 2008), *Xylocarpus*species (Z. Guo, Guo, et al., 2018), *Lumnitzera racemosa* (J. Li et al., 2016), *Bruguiera gymnorhiza* (Minobe et al., 2010; Urashi et al., 2013), *Excoecaria agallocha* (W. Guo et al., 2018),*Heritiera littoralis* (Banerjee et al., 2020), *Scaevola taccada* (Banerjee et al., 2021) and *Acanthus ilicifolus* (W. Guo et al., 2020). In this study, the Malay Peninsula was again identified to isolate populations of *A. corniculatum* . The isolation of Sundaland corresponds to the population differentiation between the subgroup "Gulf of Bengal" and "n-SCS", as well as "Bali" and "n-SCS". Despite its dominant role in mangrove species, the barrier effect of the Malay Peninsula receded largely in some coastal plants that inhabit more inland, such as *Canavalia rosea* (T. He et al., 2021) and *Pluchea indica* (Lin et al., 2020).

Genetic discontinuities in a lot of mangrove species also occur in the Wallacea region where the Indonesian-through flow functions as a major barrier hindering the dispersal of propagules. This barrier generates a population structure that populations in Southeast Asia are divergent from those in Australasia. Such pattern has been observed in the species of *R. apiculata* (Z. Guo et al., 2016), *R. stylosa* (Yan et al., 2016), *S. alba* (Y. Yang et al., 2017), *S. caseolaris*(Y. Yang et al., 2016), *Avicennia marina* (Wang et al., 2021),*C. tagal* (Huang et al., 2008, 2012), *C. decandra* (Huang et al., 2008), *X. granatum* (Z. Guo, Guo, et al., 2018), and *L. racemosa* (J. Li et al., 2016). This water barrier appears less dominant that populations of several species are not isolated, e.g.*Acanthus ilicifolus* (W. Guo et al., 2020), *H. littoralis*(Banerjee et al., 2020), *Scaevola taccada* (Banerjee et al., 2021), and *X. mucronata* (Z. Guo, Guo, et al., 2018). The difference in the ability to disperse across the sea currents may underlie the different population structures, even in congeneric species like *X. granatum* and *X. mucronata* (Z. Guo, Guo, et al., 2018). The populations of *A. corniculatum* showed a slight genetic break aligning with the Wallacea barrier. The hypocotyl of *A. corniculatum* is reported to have a relatively strong long-distance dispersal ability (Clarke, 1995).

IBD might have played a role in differentiating populations of coastal plants. Our mantel test revealed a significant positive correlation between genetic distance and geographic distance. For example, the long distance between "Bali" and "Gulf of Bengal" is likely the factor underlying their slight genetic differentiation. Taking consideration of the large geographical scale of its distribution range and the limited ability of dispersal, it's expectable to observe the IBD effect in*A. corniculatum* .

The patterns discussed above are generally consistent with previous knowledge. However, the striking genetic break between "s-SCS" and "n-SCS" is quite intriguing, some unaddressed factors should have been in function. We address this issue in the next section.

Sea-level-change-driven bottleneck enhanced population subdivision in the southern South China Sea

The genetic discontinuity between the "s-SCS" and "n-SCS" actually is not unique in *A. corniculatum.* The studies of mangrove species*E. agallocha* (W. Guo et al., 2018) and *H. littoralis*(Banerjee et al., 2020) have also shown similar genetic discontinuities. The previous studies suggested that these genetic discontinuities are attributed to the lack of suitable ocean currents to disperse fruits during the ripening season in the South China Sea (Banerjee et al., 2020; W. Guo et al., 2018). We argue that at least two observations in*A. corniculatum* are not compatible with the previous explanation. First, the differentiation between "n-SCS" and "Gulf of Bengal" is much lower than between "s-SCS" and "Gulf of Bengal". How could "n-SCS" populations exchange genes with "Gulf of Bengal" populations without bridging by "s-SCS" populations? Second, the differentiation between "s-SCS" and "n-SCS" is comparable to between "s-SCS" and "Gulf of Bengal", much higher than that between "s-SCS" and "Australasia". It's not feasible to assume that the influence of ocean currents in the SCS is even stronger than that of the Indonesian-through flow, to a level comparable with the land barrier of the Malay Peninsula. Hence, the break between the "s-SCS" and "n-SCS" populations of *A. corniculatum* cannot be simply attributed to the influence of ocean currents.

We hypothesized that bottleneck events could promote population subdivision by augmenting population differentiation. The "s-SCS" cluster was found to had undergone a bottleneck event. Our ABC simulations provided strong support that the "s-SCS" population originated from the "Australasia" cluster, with the

spit occurring at ˜1.5 Mya. The close relationship between "s-SCS" and "Australasia" was also evidenced directly by STRUCTURE and PCA clustering, and low F_{ST} values. During the split, the "s-SCS" population was reduced to a population size of ˜824, from 10538 of the common ancestral population. The drastic reduction of genetic diversity in the populations Chai-ya and Kuching evidenced the bottleneck event. The average π values in the populations Chai-ya and Kuching are only 14.3˜24.4% of those in the populations of "Australasia" subgroup and the average ϑ values are only 11.2˜25.6% (Table 2). In other words, at least 75˜90% of the ancestral polymorphisms were lost during the bottleneck event, which is consistent with the ˜92% reduction in effective population size estimated by ABC modeling.

Although the time estimation using ABC computation may not be very accurate, it roughly dated the origination of the "s-SCS" cluster at the middle Pleistocene, when glacial periods alternated with interglacial periods repeatedly. During the glacial periods (Miller et al., 2005; Voris, 2000), most Southeast Asia populations should have been wiped out. The refugial populations might have contracted to the margins of the Sundaland with a range from Wallacea to North Australia. As sea level rose in the interglacial periods, a subset of the refugia population expanded to the current southern South China Sea range as the coastline advanced (Cannon, Morley, & Bush, 2009). Such a process could have repeated multiple times. However, the genetic data obtained today are powerless to distinguish them because the ancient genetic patterns had been reshaped by more recent events.

During the bottleneck processes, intensified genetic drift and relaxed purifying selection due to reduced population size should have contributed to the loss of ancestral polymorphisms and fixation of new mutations. The observation of haplotypes unique to the "s-SCS" cluster in many genes is consistent with this interpretation. This mechanism consequently generated the current deep genetic differentiation between "s-SCS" and "n-SCS". Despite the bottleneck effect, the gene flow between "s-SCS" and "n-SCS" seems not completely blocked, evidenced by the occurrence of "n-SCS" haplotypes with relatively high frequency in "s-SCS" populations. Our ABC modeling also supported the existence of such gene flow. In contrast, gene flow in the reciprocal direction is much lower, indicated by that "s-SCS" haplotypes were rarely observed in "n-SCS" populations.

As mentioned before, the substantial genetic break between "s-SCS" and "n-SCS" has also been observed in *E. agallocha* (W. Guo et al., 2018) and *H. littoralis* (Banerjee et al., 2020), which may also be attributed to the mechanism we described above. However, the mismatch distribution analyses presented in the original papers provided no support for a sudden expansion model (Banerjee et al., 2020; W. Guo et al., 2018). Notably, obvious differentiation has been observed between the populations sampled from Northeastern Borneo and surrounding populations in *S. alba* (Y. Yang et al., 2017) and *A. marina* (Wang et al., 2021). Such population structures in the smaller geographical ranges are highly likely generated by a bottleneck process. Further studies may test this hypothesis in these species. In contrast, the populations of some mangrove species, whose distribution range covers both the northern and southern parts of the South China Sea, are found to be genetically continuous with confidential data. Such species include but may not limit to *R. apiculata* (Z. Guo et al., 2016),*R. stylosa* (Yan et al., 2016), *C. decandra* (Huang et al., 2012), *B. gymnorhiza* (Urashi et al., 2013) and *L. racemose*(J. Li et al., 2016). Comparing to those genetic structures attributed to geographical barriers, the population structure generated by bottleneck events appears to be rarer and more unpredictable.

Demographic size dynamics such as bottleneck may be a general mechanism to shape the genetic structure of coastal plants

Consistent with our hypothesis, we observed that the bottleneck event caused by past sea-level changes had promoted population subdivision in*A. corniculatum* , resulting in the genetic break between populations in the southern and northern South China Sea. This mechanism is not likely unique to *A. corniculatum* , but generally to other species. The intraspecific genetic diversity of *Senecio rodriguezii* was found to be highly structured in that cpDNA haplotypes were not shared between the Mediterranean islands and a high proportion of haplotypes were restricted to small geographical areas within the islands (Molins, Mayol, & Rosselló, 2009). The population history of this species was supposed to have been dominated by both

expansion and contraction events in the Quaternary sea-level changes. The three major lineages of *Nigella arvensis* in the Aegean Archipelago were found to evolve from multiple fragmentation events of a widespread ancestral stock, in which genetic drift appears to have played a significant role (Bittkau & Comes, 2005).

The mechanism of bottleneck events promoting population subdivision is highly likely applicable to coastal plants, which are common to have buoyant propagules, even in species growing on coastal dunes which are rarely inundated by seawater (Kadereit et al., 2005; H. Yang et al., 2012). Due to the nature of dispersing via seawater, genetic discontinuities in coastal plants were usually correlated with geographical barriers of land masses and ocean currents. It has known that biological properties of a species, such as breeding system, dispersal ability, and life history, would have an influence on how the geographic forces shape genetic variation within its distribution range (Nybom & Bartish, 2000; Wee et al., 2020; Westberg & Kadereit, 2009). We showed that the high dynamics of the habitats of coast plants may introduce additional complexity to their phylogeography.

The demographic histories of some coastal plants in the IWP region have been investigated. The effective population size (N_e) of *R. apiculata* populations in the Malacca Strait (Z. Guo et al., 2016) and *L. racemosa* populations in the Malacca Strait and Hainan island were found to have been drastically reduced (J. Li et al., 2016). In contrast, the *S. alba* populations in the Malacca strait have largerN_e than the surrounding populations, due to population admixture (Y. Yang et al., 2017). The mismatch distribution analysis and neutrality tests revealed some signals of population expansion in *A. ilicifolius* (W. Guo et al., 2020) while the BOTTLENECK analysis revealed no evidence of genetic bottleneck in*Canavalia rosea* (T. He et al., 2021). Interestingly, the possible correlation between genetic structure and historic demographic size change was not explored in these studies. Even if the mechanism we proposed before is general in the natural world, a reliable investigation into the bottleneck event must precede correlating it to any observed genetic break.

Lastly, attention to the demographic size change of a species is of particular importance for conservation. A large population is usually the fundament for plants to provide ecological services. The ecological importance of mangrove species has never been overly emphasized. Investigating their historic effective population sizes is an efficient approach to retrospect their past and prospect their future response to the current global change (Z. Guo, Li, et al., 2018). The *A. corniculatum* populations in the southern South China Sea call for conservation priority due to the reduced genetic diversity.

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DATA ACCESSIBILITY

GenBank accession numbers of reference sequences for the genes are KF745073, KF745075- KF745085, KF745087- KF745088, KF745090, KF745092, KF745095- KF745097, KF745099- KF745106, KF745108- KF745111 KF745113- KF745125, KF745127- KF745130, KF745132- KF745139, KF958338, KF958340, KF958343- KF958345, and KF977861-KF977866 (the detailed information could be found in the supplementary Table S2 of He et al 2019, doi: 10.1093/nsr/nwy078). The raw reads of Illumina sequencing data were deposited to Genome Sequence Archive in National Genomics Data Center (accession number CRA004437). The Sanger sequences of population individuals were deposited to NCBI (accession number PRJNA739874)

AUTHOR's CONTRIBUTIONS

Z. G and S. S conceived the study. S. S., L. F., C. Z., and N. D. collected the samples. R. Z., Z. G., and L. F. analysed the data. Z. G., R. Z. and S. S. wrote the manuscript. All authors read and approved the manuscript.

SUPPORTING INFORMATION

The online supplementary file contains Table S1-S3 and Figure S1-S9.

Table S1 Prior distributions of model parameters in the approximate Bayesian computation. **Table S2** Summary information of 84 haplotype segments length larger than 300 bps. **Table S3**Analysis of molecular variance (AMOVA) of the *Aegiceras corniculatum* populations from the Indo-West Pacific basing on the six Sanger-sequenced genes. **Figure S1** Optima K of all 18 Sanger-sequenced populations obtained by the Evanno's method.**Figure S2** Optima K of "Indo-Malayan" group obtained by the Evanno's method. **Figure S3** Optima K of "Pan-Australia" group obtained by the Evanno's method. **Figure S4** Heatmap of pairwiseF_{ST} values of *Aegiceras corniculatum*populations, which were obtained from the Illumina data. **Figure S5** Heatmap of pairwise D_{XY} values of*Aegiceras corniculatum* populations obtained from the Sanger data.**Figure S6** Heatmap of pairwise D_{XY} values of Aegiceras corniculatum populations, which were obtained from the Illumina data. **Figure S7** Mantel test on all *Aegiceras corniculatum* populations based on F_{ST} matrices from Illumina data. **Figure S8** Mantel test on the*Aegiceras corniculatum* populations of "Pan-Australasia" group based on the F_{ST} matrices from the Sanger data.**Figure S9** Mantel test on the *Aegiceras corniculatum*populations of "Indo-Malayan" group based on theF_{ST} matrices from the Sanger data.

Table 1. Basic information of population sampling of*Aegiceras corniculatum* .

Site ID	Location	Longitude and Latitude	Sequencing platform	Sample size +	
Sanya	Sanya, Hainan, China	18°14'N, 109°30'E	Sanger & Illumina	24(24)	24(24)
Wenchang	Wenchang, Hainan, China	19°36'N, 110°47'E	Sanger & Illumina	24(100)	
Yalong	Yalong Bay, Hainan, China	18°13'N, 109°36'E	Sanger & Illumina	24(50)	
U-Daintree	Upper Daintree River, Australia	16°16'S, 145°18'E	Sanger & Illumina	24(40)	
Darwin	Darwin, Australia	12°28'S, 130°51'E	Sanger & Illumina	24(42)	
Sorong	Sorong, West Papua, Indonesia	0°52'N, 131°15'E	Sanger & Illumina	22(22)	
La-un	La-un, Thailand	10°10'N, 98°43'E	Sanger & Illumina	33(36)	
Ngao	Ngao, Thailand	09°52'N, 98°36'E	Sanger & Illumina	37(51)	
Kuching	Kuching, Malaysia	1°34'N, 110°21'E	Sanger & Illumina	23(40)	
Chai-ya	Chai-ya, Thailand	9°23'N, 99°16'E	Sanger & Illumina	24(50)	
Bali	Bali, Indonesia	8°43'S, 115°19'E	Sanger & Illumina	24(36)	

Site ID	Location	Longitude and Latitude	Sequencing platform	Sample size +
Danzhou	Danzhou, Hainan, China	19°26'N, 109°34'E	Sanger	24
Dongzhai	Dongzhai harbor, Hainan, China	19°58'N, 110°34'E	Sanger	27
Bangladesh	Bangladesh	22°1'N, 89°46'E	Sanger	22
Pambala	Pambala, Sri Lanka	7°30'N, 79°50'E	Sanger	11
Rekawa	Rekawa, Sri Lanka	6°3'N, 80°51'E	Sanger	10
Sibu	Sibu, Malaysia	6°2'N, 116°7'E	Sanger	22
L-Daintree	Lower Daintree River, Australia	16°17'S, 145°27'E	Sanger	24

+ the numbers outside brackets are individual number for Sanger sequencing while those inside brackets are number for Illumina sequencing.

Table 2. Genetic diversity estimation for 18 populations of*Aegiceras corniculatum* .

Location ID	Analysed length (depth>100bp)	Genes	SNPs	Polymorphic genes	$\vartheta(\times 10^{-3})$	$\pi(\times 10^{-3})$
Kuching	71655	77	132	32	0.461	0.494
Chai-ya	72314	78	91	11	0.298	0.412
La-un	63098	69	639	65	2.491	2.703
Ngao	71689	76	619	65	2.032	2.109
Sanya	64323	75	209	37	0.893	0.834
Wenchang	75214	82	524	59	1.507	1.347
Yalong	74568	82	260	39	0.867	0.892
U-Daintree	70890	77	761	76	2.652	2.885
Darwin	72515	79	656	76	2.147	2.574
Sorong	74228	81	479	67	1.798	2.023
Bali	73564	78	291	50	0.944	1.208
Danzhou	4494	6	39	6	1.973	2.948
Dongzhai	4494	6	37	6	1.891	2.871
Bangladesh	4494	6	26	5	1.373	1.921
Pambala	4494	6	17	3	1.041	1.076
Rekawa	4494	6	16	3	1.029	1.498
Sibu	4494	6	33	2	1.788	3.259
L-Daintree	4494	6	37	6	1.926	2.284

Table 3. Posterior probabilities of models using approximate Bayesian computation

	Repeat 1	Repeat 2	Repeat 3
Model 1	0.0000	0.0000	0.0015
Model 2	0.0000	0.0000	0.0018
Model 3	0.0002	0.0000	0.0073
Model 4	0.0019	0.0004	0.0114
Model 5	0.0011	0.1878	0.1189
Model 6	0.2851	0.0449	0.0507
Model 7	0.0320	0.0290	0.0394
Model 8	0.6332	0.7109	0.6803
Model 9	0.0000	0.0009	0.0048
Model 10	0.0048	0.0020	0.0079
Model 11	0.0028	0.0017	0.0141

	Repeat 1	Repeat 2	Repeat 3
Model 12	0.0389	0.0225	0.0619

Table 4. Estimates of the principal demographic parameters for the best supported ABC models

Parameters	Ne	T_0 (myr)	T_1 (myr)	T_2 (myr)	m_1	m_2	m_3	m_4	Bottleneck
Mode	1917	2.711	1.493	0.127	0.258	0.007	0.005	0.403	0.427
95% CI (lower) +	1812	2.585	1.236	0.104	0.118	0.002	0	0.216	0.18
95% CI (upper)	1986	3.014	1.858	0.206	0.688	0.021	0.075	0.775	0.485

+ 95% CI indicates the 95% confidence intervals.

*Ne*represents the number of diploid individuals in the population.

T_0 represents the time the "Indo-Malayan" lineage and the ancestor of the "Australasia" and "s-SCS" lineages diverged. The unit of time is million years.

T_1 represents the time the "s-SCS" lineage diverged from the "Australasia" lineage. The unit of time is million years.

T_2 represents the time the N_e of the "s-SCS" lineage increased. The unit of time is million years.

m_1, m_2, m_3, and m_4 represent the gene flow from the "Indo-Malayan" to the "s-SCS" populations, from the "s-SCS" to the "Indo-Malayan" populations, from the "s-SCS" to the "Australasia" populations, and from the "Australasia" to the "s-SCS" populations, respectively.

bottleneck represents the proportion of *Ne* reduced.

Figure 1. Population genetic diversity of *Aegiceras corniculatum* . (a) Distribution of π and ϑ across 18 populations (per kb). The height of the rectangle is proportional to the levels of π and ϑ. Blue is π, and red is ϑ. Dark red/blue bar indicate value obtained from Illumina data while light red/blue ones indicate values obtained from Sanger data. (b) Boxplots of the π of each gene across 11 Illumina-sequenced populations (per kb). (c) Boxplots of the ϑ of each gene across 11 Illumina-sequenced populations (per kb). Boxes of the same colour indicate populations in the same geographical area. "s-SCS", "n-SCS", "Gulf of Bengal" and "Australasia" indicate geographical regions. "Total" indicates the genetic divergence of*Aegiceras corniculatum* at the species level.

Figure 2. Genetic differentiation and structure of*Aegiceras corniculatum* populations. (a) Heatmap of the pairwiseF_{ST} values of the 18 populations estimated from the six Sanger-sequenced genes. (b) Plot of the first and second axes of a principal component analysis based on the frequency of single nucleotide polymorphisms within each population of 82 deep sequencing genes. The letters are the site ID of the sampled populations. The populations in different clusters are indicated by different colours. (c) Bayesian clustering with STRUCTURE split the 18 populations of*Aegiceras corniculatum* into two groups, and each group was further clustered into 3 (4) or 2 subgroups. Each thin vertical bar represents an individual, and each bold vertical bar separated by a black line represents a population. The population names are listed below the plot.

Figure 3. Geographical distribution patterns of the haplotypes inferred in the 6 Sanger-sequenced genes. The corresponding haplotype networks are drawn in the lower left corners of the maps; the colours corresponded to the haplotypes. In the haplotype network, the circle size is approximately proportional to the number of individuals of each haplotype. The number beside the lines indicates the mutation steps. The representative gene segment ID of each pattern is written to its upper right.

Figure 4. Six different geographical distribution patterns of the haplotypes inferred from the 41 Illumina-sequenced genes. The corresponding haplotype network is drawn in the upper right corner of each map; the colours correspond to the haplotypes. In the haplotype network, the circle size is approximately proportional to the number of individuals of each haplotype. The small black nodes on the lines are the median vectors, indicating the hypothesized (often ancestral) sequences required to connect the existing sequences within the network with maximum parsimony. The number beside the lines indicates the mutation steps. The representative gene segment ID of each pattern is written to its lower left.

Figure 5. Geographical factors underlying the genetic structure of *Aegiceras corniculatum* revealed by BARRIER inference and Mantel test. (a) Results of the barrier analysis (Manni et al., 2004) based on deep-sequencing data, showing the spatial separation of*A. corniculatum* populations. The detected barriers (thick coloured lines) separate 11 populations into five groups. Green, blue, orange and purple lines correspond to the first, second, third and fourth barriers. The thickness of a coloured line indicates the number of F_{ST} matrices (black number adjacent to the line) that support the corresponding barrier. (b) Mantel test based on the F_{ST} matrices of six Sanger-sequenced genes. Different types of population pairs were indicated with different marks and colours.

Figure 6. Twelve models simulating demographic history. Blue indicates Lineage 1, which includes Kuching and Chai-ya. Orange indicates Lineage 2, which includes U-Daintree, Darwin, and Sorong. Green indicates Lineage 3, which includes Sanya, Wenchang, Yalong, La-un, Ngao and Bali. The double-sided arrow indicates migration. T_0-T_2 indicate the time points of historic events and m_1-m_4 indicate gene flows.