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A new computational method to estimate adaptation time in *Avicennia* by using divergence time

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Evolutionary studies of plant groups which are distributed in vast geographical regions and face different ecological and environmental conditions are important as they through light on different mechanisms of local adaptation and species divergence through time. The genus *Avicennia* is one of these plant groups which inspire of few species show interesting geographical distribution with some degree of species-specific geographical isolations. In general, very limited molecular phylogenetic investigations have been carried out in the genus *Avicennia*, and therefore we conducted the present study with the following aims:

1. To estimate the species divergence time based on different nuclear and chloroplast DNA regions, separately. This will illustrate how different genetic regions evolved in this genus,

2. To identify the sequences with potential adaptive value against geographical variable by latent factor mixed models (LFMM) analysis,

3. To illustrate the phylogenetic signal of these DNA regions and their role in speciation within the genus and,

4. To introducing a new computational strategy for estimating adaptive time for the sequences. The results showed that different genetic regions may produce different species divergent time, both the nuclear ribosomal internal transcribed spacer (ITS) region and chloroplast DNA sequences, contained potentially adaptive single nucleotide polymorphisms (SNPs).We could present a suggestive time for these adaptive sequences for the first time. In conclusion both local adaptation and independent mutations seem to have played role in *Avicennia* speciation and evolution.

Keywords Adaptive time, *Avicenna*, Divergence time, LFMM, SNPs

The genus *Avicennia* is composed of about eight species of mangrove trees which grow in intertidal zones in tropical and temperate regions of the world. *Avicennia* has developed several unique features, such as salt glands, aerial and prop roots, finger-like pneumatophores, and crypto viviparous fruits with the ability to float in salt water and therefore, occupy the newly formed seaward mangrove habitats on tropical coasts[1]. These plant species are economically important and are utilized as medicinal plants for treatment of various diseases such as cancer, diabetes, malaria, rheumatism, asthma, small pox and ulcer[2].

The eight species of *Avicennia* are namely: *A. germinans* (L.) Stearn, *A. schaueriana* Stapf & Leechm. ex Moldenke, *A. bicolor* Standley, *A. marina* (Forssk.) Vierh, *A. alba* Blume, *A. officinalis* L., *A. integra* N. C. Duke, and *A. rumphiana* Hallierf[3]. Geographical distribution of the first three species is the Atlantic-East Pacific (AEP) region; while, the other five species live in the Indo-West Pacific region (IWP)[3]. *A. marina*, has a broad geographical distribution within IWP and contains three infraspecific varieties[3].

A recent molecular phylogenetic investigation[1] took into account the species evolutionary relationships and divergence time of this genus based on a combined data set of different chloroplast and nuclear genes for a few representatives of *Avicennia* species. However, our recent studies concerned with different evolutionary aspects of the genus revealed that these species show variation due to vast geographical distribution and introgressive hybridization and also different genetic segments within both chloroplast and nuclear genes, have different adaptive potentials.

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1

It has been suggested change in environments, drives the geographical populations towards a fast adaptation repeatedly and even at micro-geographic scales. These rapid parallel evolutions are supported by standing genetic variation, particularly those sequences with potential adaptive value[4]. However, due to subsequent admixture as it is frequent among *Avicennia* species and populations, the ancient and differentially selected alleles become polymorphic in most populations across its range, which in turn may allow for the fast evolution of one ecotype from a small number of random individuals, from a population of the other ecotype. In general, the cases of fast parallel ecological divergence as also encounters in *Avicennia* can be the result of evolution at two different time frames: divergence in the past, followed by repeated selection on the same divergently evolved alleles after admixture[4].

Therefore, we carried out the present study with the following tasks: 1- Estimating divergence of the *Avicennia* species, based on individual chloroplast and nuclear ITS genes, 2- Identify the genes sequences which have a potential adaptive value against geographical variables and, 3- Provide a probable date for these adaptive sequences. For these studies we used multiple computational approaches like, Bayesian-based LFMM, and divergence time estimation as implemented in BEAST 2 (Bayesian Evolutionary Analysis by Sampling Trees). Moreover, we are introducing a new approach for estimating probable time for adaptation of the nucleotides.

Materials and methods
Sequence retrieval
Nuclear ITS and chloroplast DNA (trnH-psbA and trnL-F) sequences of *Avicennia* species were obtained and aligned by MUSCLE program in Molecular MEGA (Molecular Evolutionary Genetics Analysis) 11 software (Tamura et al.2021). The sequences recorded at National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/) (Tables 1, 2, 3). In total, 53 ITS, 31 trnH-psbA, and 21 trnL- F sequences were used for analyses.

Species divergence time estimation
Nuclear ITS sequences were aligned by MUSCLE program in MEGA 7 software. We used BEAST 2.4.8[6] for the Bayesian MCMC inferred analyses of the nucleotide sequence data. BEAUti 2 (Bayesian Evolutionary Analysis Utility version)[6] was utilized to generate initial xml files for BEAST. A Yule process of speciation ('a pure birth' process) was used as a tree prior for all the tree model analyses.

The Yule tree prior is widely recognized as giving the best-fit model for trees describing the relationships between different species. The parameter can be regarded as explaining the net speciation rate[7]. For the MCMC posterior analyses, the length of chain was 10 000 000. After 100 trees burn-in processing, 10 000 trees were used for the analyses.

The BEAUti xml file was run in the BEAST 2.4.8 program and the maximum clade credibility (MCMC) chain generations were repeated five times for each molecular clock model with independent runs to ensure suitable convergence and adequate mixing.

The MCC tree was generated under the relaxed clock model and with HKY substitution. Tracer v1.5 software[8] was used for the output of the model parameters to examine the sampling and convergence results obtained from BEAST. Tree Annotator v1.6.1 software[9] was used to annotate the phylogenetic results generated by BEAST as a form of single 'target' tree. We used the Figtree v1.3.1[10] to present the chronicle tree constructed. The posterior probability was set to 0.5, which is equivalent to the bootstrapping value. A TCS network as implemented in POPART program was also used to illustrate the split time in the studied species.

Identification of SNPs with adaptive potentials
For revealing and identifying the SNPs within trnL-F and trnH-psbA genetic regions, we used both RDA (Redundancy analysis), as well as LFMM (Latent factor mixed model). We used ITS data of the same analyses we had obtained from our previous study. RDA is a form of constrained ordination that suits for genomic data sets, where we are interested in understanding how the multivariate environmental factors shape the patterns of genomic composition across geographical areas. RDA is based on multivariate regression, and takes in to account a model of linear combinations of the environmental predictors that explain linear combinations of the SNPs. This method effectively identifies covering loci associated with the multivariate geographical variables[11]. RDA was performed in PAST ver. 4, program[12].

LFMM is a Bayesian approach method for testing associations between loci and geographical gradients using latent factor mixed models. It performs a regression analysis in which the confounding variables are modeled with unobserved (latent) factors. The program estimates correlations between geographical variables and allelic frequencies, and simultaneously infers the background levels of population structure[13,14]. LFMM was performed by LFMM package in R. 4.2.

Estimating the adaptive SNPS time of adaptation
For suggesting a probable time for adaptive SNPs in each gene segment, we kept only the potentially adaptive SNPS and performed BEAST analysis for divergence time. A change in date obtained from whole SNP data to adaptive SNP data is possibly suggesting the time for SNP adaptation. We used the same procedure as stated for the species divergence time estimation in BEAST 2.4.8. The following formula is introduced by us for estimating an adaptive time for the nucleotides.

Sp	Accession number
Avicennia alba	EF540977.1
Avicennia alba	AF365980.1
Avicennia alba	MH243937.1
Avicennia alba	MH243936.1
Avicennia alba	MH243935.1
Avicennia alba	MH243934.1
Avicennia alba	MG880036.1
Avicennia alba	MG880035.1
Avicennia alba	MG880034.1
Avicennia alba	MG880033.1
Avicennia alba	MG880032.1
Avicennia alba	MG880031.1
Avicennia alba	MG880030.1
Avicennia alba	MG880029.1
Avicennia alba	MG880028.1
Avicennia alba	EU528876.1
Avicennia alba	KX641594.1
Avicennia alba	KJ784551.1
Avicennia alba	KF848261.1
Avicennia bicolor	EF540988.1
Avicennia bicolor	EF540988.1
Avicennia bicolor	EF540987.1
Avicennia bicolor	EU352151.1
Avicennia bicolor	EU352150.1
Avicennia bicolor	EU352149.1
Avicennia bicolor	AF365977.1
Avicennia bicolor	EU528877.1
Avicennia germinans	EF540990.1
Avicennia germinans	EF540985.1
Avicennia germinans	EF540984.1
Avicennia germinans	EF540983.1
Avicennia germinans	EF540982.1
Avicennia germinans	EF540981.1
Avicennia germinans	EF540980.1
Avicennia germinans	EU352146.1
Avicennia germinans	EU352147.1
Avicennia germinans	KX641596.1
Avicennia germinans	MG880047.1
Avicennia germinans	MG880046.1
Avicennia germinans	MG880045.1
Avicennia germinans	MG880041.1
Avicennia germinans	MG880040.1
Avicennia germinans	MG880039.1
Avicennia germinans	MG880038.1
Avicennia germinans	MG880037.1
Avicennia germinans	DQ469846.1
Avicennia germinans	DQ469845.1
Avicennia germinans	DQ469860.1
Avicennia germinans	DQ469859.1
Avicennia germinans	DQ469858.1
Avicennia germinans	DQ469857.1
Avicennia germinans	DQ469856.1
Avicennia germinans	DQ469855.1
Avicennia germinans	DQ469854.1
Avicennia germinans	DQ469853.1
Avicennia germinans	DQ469852.1
Continued	

Sp	Accession number
Avicennia integra	KX641598.1
Avicennia officinalis	MH243949.1
Avicennia officinalis	MH243948.1
Avicennia officinalis	MH243947.1
Avicennia officinalis	MH243946.1
Avicennia officinalis	MH243945.1
Avicennia officinalis	MH243944.1
Avicennia officinalis	MH243943.1
Avicennia officinalis	MG880054.1
Avicennia officinalis	MG880053.1
Avicennia officinalis	MG880052.1
Avicennia officinalis	MG880051.1
Avicennia officinalis	MG880050.1
Avicennia officinalis	KX641597.1
Avicennia officinalis	KJ784553.1
Avicennia officinalis	KF848263.1
Avicennia rumphiana	KX641595.1
Avicennia schaueriana	EF540986.1
Avicennia schaueriana	DQ469862.1
Avicennia schaueriana	AB861412.1
Avicennia schaueriana	AB861385.1
Avicennia schaueriana	AB861382.1
Avicennia schaueriana	AB861365.1
Avicennia schaueriana	AB861357.1
Avicennia schaueriana	AB861354.1
Avicennia schaueriana	AB861345.1
Avicennia schaueriana	AB861327.1
Avicennia schaueriana	AB861326.1
Avicennia schaueriana	AB861325.1
Avicennia schaueriana	AB861307.1
Avicennia schaueriana	AB861306.1
Avicennia schaueriana	AB861305.1
Avicennia schaueriana	AB861287.1
Avicennia schaueriana	AB861286.1
Avicennia schaueriana	AB861285.1
Avicennia schaueriana	AB861284.1
Avicennia schaueriana	AB861280.1
Avicennia schaueriana	AB861270.1
Avicennia schaueriana	AB861266.1
Avicennia schaueriana	AB861265.1
Avicennia schaueriana	AB861263.1
Avicennia schaueriana	AB861257.1
Avicennia schaueriana	AB861251.1
Avicennia schaueriana	AB861246.1
Avicennia schaueriana	AB861245.1
Avicennia schaueriana	AB861244.1
Avicennia schaueriana	AB861240.1
Avicennia schaueriana	AB861231.1
Avicennia schaueriana	AB861226.1
Avicennia schaueriana	AB861225.1
Avicennia schaueriana	AB861224.1
Avicennia schaueriana	AB861222.1
Avicennia schaueriana	AB861220.1
Avicennia marina	MF063712.1
Avicennia marina	MF063711.1
Avicennia marina	MF063710.1
Continued	

Sp	Accession number
Avicennia marina	MF063709.1
Avicennia marina	MF063708.1
Avicennia marina	EF540978.1
Avicennia marina	AF477771.1
Avicennia marina	AF477770.1
Avicennia marina	MN883387.1
Avicennia marina	MN883386.1
Avicennia marina	MN883385.1
Avicennia marina	MN883384.1
Avicennia marina	MH243942.1
Avicennia marina	MH243941.1
Avicennia marina	MH243940.1
Avicennia marina	MH243939.1
Avicennia marina	MH243938.1
Avicennia marina	MG880049.1
Avicennia marina	MG880048.1
Avicennia marina	MK027295.1
Avicennia marina	EU528879.1
Avicennia marina	KM652500.1
Avicennia marina	KF848262.1
Avicennia marina	DQ469861.1
Avicennia marina subsp. *marina*	KX641593.1
Avicennia marina subsp. *Eucalyptifolia*	KX641592.
Avicennia marina subsp. *australasica*	KX641591.
Avicennia marina subsp. *australasica*	AF365978.1

Table 1. Voucher information and GenBank accession numbers of taxa sampled for the genus *Avicennia* based on ITS data.

SP	Accession number
Avicennia officinalis	KT161361.1
Avicennia officinalis	MN117565.1
Avicennia officinalis	MN117564.1
Avicennia officinalis	MN117563.1
Avicennia officinalis	MN117562.1
Avicennia officinalis	MN117561.1
Avicennia officinalis	MN117560.1
Avicennia officinalis	MN117559.1
Avicennia alba	MN117553.1
Avicennia alba	MN117552.1
Avicennia alba	MN117551.1
Avicennia alba	MN117550.1
Avicennia alba	MN117549.1
Avicennia alba	JX448690.1
Avicennia marina	KT161360.1
Avicennia marina	MN117558.1
Avicennia marina	MN117557.1
Avicennia marina	MN117556.1
Avicennia marina	MN117555.1
Avicennia marina	MN117554.1
Avicennia marina	JX448688.1
Avicennia marina	MF064829.1
Avicennia marina	MF064830.1
Avicennia marina	MF064831.1
Avicennia marina	MF064832.1
Avicennia marina	MF064833.1
Avicennia germinans	KC420634.1
Avicennia germinans	KJ426610.1
Avicennia germinans	HG963703.1
Avicennia bicolor	KC420633.1
Avicennia rumphiana	JX448689.1

Table 2. Voucher information and GenBank accession numbers of taxa sampled for the genus *Avicennia* based on trnh-psba data.

Sp	Accession number
Avicennia officinalis	KT074999.1
Avicennia officinalis	MH215695.1
Avicennia officinalis	MH215694.1
Avicennia officinalis	MH215693.1
Avicennia officinalis	MH215692.1
Avicennia officinalis	MH215691.1
Avicennia officinalis	MH215690.1
Avicennia officinalis	MH215689.1
Avicennia alba	MH215683.1
Avicennia alba	MH215682.1
Avicennia alba	MH215681.1
Avicennia alba	MH215680.1
Avicennia alba	MH215679.1
Avicennia marina	KT074998.1
Avicennia marina	MH215688.1
Avicennia marina	MH215687.1
Avicennia marina	MH215686.1
Avicennia marina	MH215685.1
Avicennia marina	MH215684.1
Avicennia marina	KM888791.1
Avicennia marina	JQ728990.1

Table 3. Voucher information and GenBank accession numbers of taxa sampled for the genus *Avicennia* based on *trnL-F* data.

$\hat{At}$ = Suggested approximate time of adaptation for nucleotides

$$\hat{At} = Ln\left(2^{-\Delta\Delta Dt}\right)$$

$$2^{-\Delta\Delta Dt} = \overline{\Delta Dt}_1 - \overline{\Delta Dt}_2$$

Dt_{1i}^{1-N} = Divergence time for each sample/ species

$\overline{\Delta Dt}_1$ = The mean Divergence time difference of the samples Based on total sites

$$\overline{\Delta Dt}_1 = \sum Dt_{1i} \Big/ N \qquad (N = \text{Number of Samples/ species})$$

Dt_{2i}^{1-N} = Divergence time for each sample/ species based on adaptive sites

$\overline{\Delta Dt}_2$ = The mean Divergence time difference of the samples based on adaptive sites

$$\overline{\Delta Dt}_2 = \sum Dt_{2i} \Big/ N$$

Determination of phylogenetic role of adaptive sequences

To determine the phylogenetic role of adaptive sequences we used phylogenetic PCA (pPCA) approaches as suggested by Ravell[15] and also Jombart[16]. For this we used the species phylogenetic tree obtained from the whole sequence data and then performed character mapping of adaptive nucleotides on this phylogenetic tree.

Results

Divergence time estimation

BEAST chronological tree of *Avicennia* species based on ITS sequences is presented in Fig. 1. In general, the species diverged from almost 1–4 MYA. The TCS network (Fig. 2), shows the probable split time of the studied taxa. Divergence estimates within the IWP clade, viz. the *A. alba*, *A. officinalis*, and *A. marina*, took place around 1.6–1.8 MYA. Similarly, the split time within Atlantic-East Pacific (AEP) lineage, viz. *A. schuaueriana*, *A. bicolor*, and *A. germinans*, occurred between 1 and 3.5 MYA.

The chronological phylogenetic tree of *Avicennia* species based on trnH-psbA sequences is provided in Fig. 3. The divergence time obtained is relatively smaller for this gene compared to that of ITS sequences. The trnH-psbA sequences suggest a divergence time of 1.2–1.5 MYA, for *Avicennia* species.

The BEAST chronological tree of *Avicennia* species based on trnL-F sequences, produced a time of divergence almost close to that of ITS sequence data (Fig. 4). These results show that different genetic segments may portrait a different time of species divergence in the genus *Avicennia*, and that these different genetic sequences may show different evolutionary pathway and response to environmental variables. The following analyses carried out by LFMM method produce insight into the sequences of these genetic regions which have a potential adaptation value.
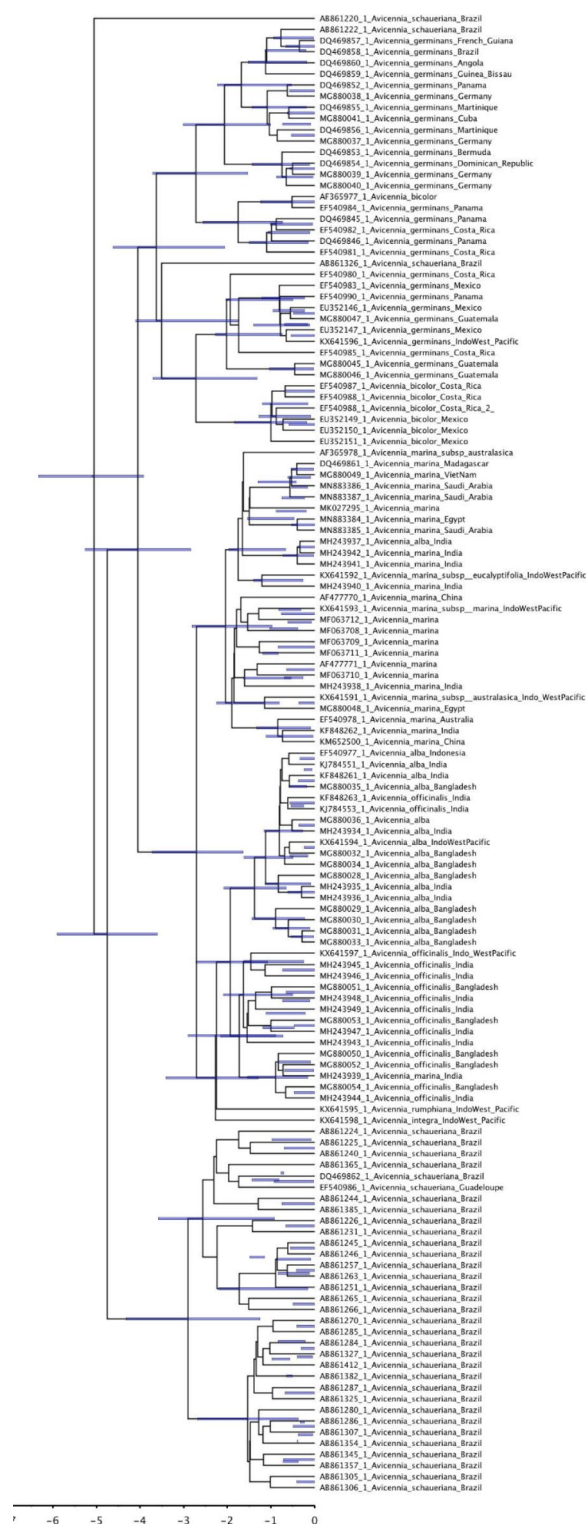
Fig. 1. BEAST chronological tree showing probable divergence time for *Avicennia* species based on ITS sequence data.

Identifying adaptive SNPs

LFMM based p-values of each nucleotide for 115 ITS nucleotides were obtained after alignment and curation of data of these only 16 sites showed significant association with geographical variables (colored SNPs). These are SNPS NUMBER 11, 36, 41, 42, 51, 54, 56, 82, 100, 103, 195, 107, 109, 110, and 114.

A similar analysis for trnH-psbA and trnL-F sequences also identified the nucleotides with potential adaptive value against geographical variables. The Manhattan plot of the LFMM analysis for trnH-psbA is provided in
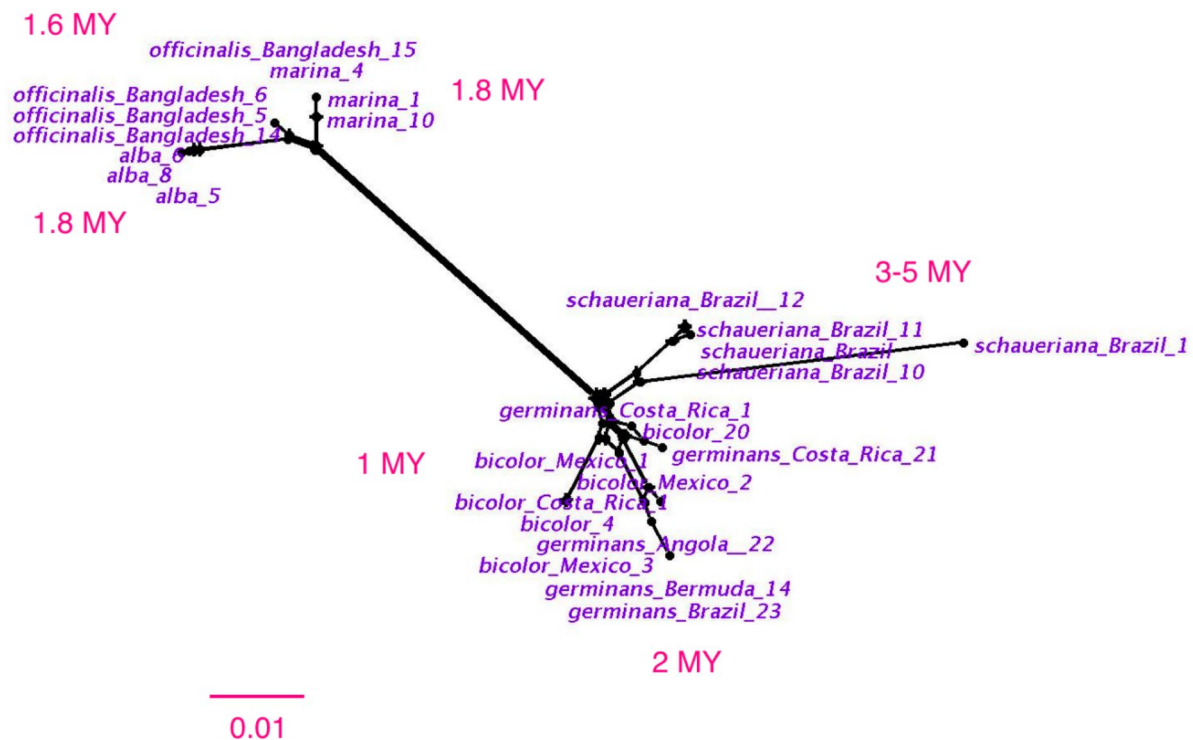
Fig. 2. TCS network of ITS sequences showing divergence time within different *Avicennia* clades.

Fig. 5. Out of 86 nucleotides, about 12 SNPs are significantly associated with geographical variables. We obtained no significant association between trnL-F sequences and geographical variables.

These results indicate that at least some of the nucleotides within ITS and trnH-psbA genetic regions were under local selection and were adapted to geographical variables. To produce data on probable date of the sequences' adaptation, we first carried out divergence time estimation based on merely adaptive nucleotides which were identified by LFMM analysis. This new divergence time estimate would be run against the all sequence data divergence time obtained. The difference in their time (Delta divergence time), may suggest the time past for sequences to be adapted.

BEAST chronological tree of the studied taxa, based on merely adaptive ITS nucleotides, is provided in Fig. 6. As it is evident from this tree, the divergence time has been relatively older that all sequence data. That is, the mean time for total sequences was about 4 MY, while the same time for only adaptive nucleotides goes back to 6 MY. The Ln value of delta time may be related to the time past for adaptation of these sequences through local selective pressure.

Therefore, if we consider the mean dt1 time (Divergence time for total sequences) = 4 MY, and the mean Dt2 time (Divergence time based on adaptive sequences) = 6, the Ln of delta time i.e. suggested adaptive time (At) = 0.7 MY. That means about 0.7 MY time took place for ITS sequences' adaptation.

A similar analysis for trnH-psbA sequences produced the mean time for total sequences is about 1 MY, while the same time for only adaptive nucleotides reaches to 3.5 MY. The delta time = 2.5 MY, and therefore at = Ln (-2.5) = 0.9 MY(Fig. 7).

This indicates that trnH-psbA sequences took relatively a longer time to be selected for adaptation, compared to that of ITS sequences in *Avicennia* species. This longer time may be related to the nature trnH-psbA chloroplast genome which is more variable and has a higher magnitude of mutations / nucleotide substitutions, compared to that of ITS sequences with a more conservative nature. Since we found no sequence in trnL-F region adapted to geographical variables, we did not carry out the above said analyses for these sequences.

Phylogenetic signal of adaptive sequences

Phylogenetic PCA (pPCA), of ITS data is provided in Fig. 8 of these adaptive nucleotides, SNP number 36, 41, 100, 109, and 114 shows a phylogenetic signal as they are present in *A. alba*, *A. officinalis*, and *A. marina*, which comprise a distinct phylogenetic clade.

Moreover, some of these SNPs also shows close relationship between *A. rumphiana* and the above said clade. A similar analysis of trnH-psbA adaptive nucleotides (Fig. 9), revealed that the SNPs number 1, 3, 8, 9, 11, and 12 have some degree of phylogenetic signal as they show species relationship within a sub-clade of the tree.
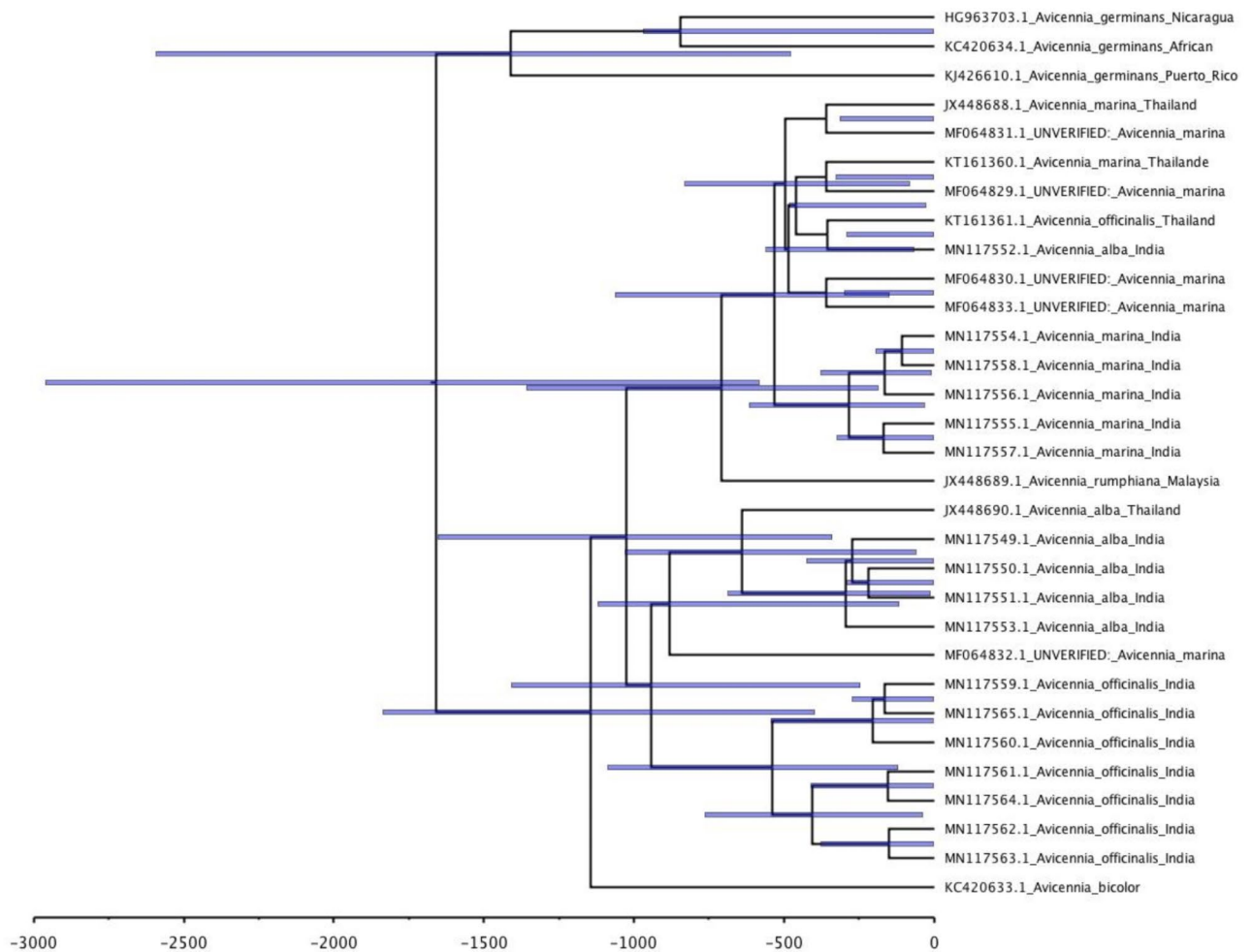
Fig. 3. BEAST chronological tree of *Avicennia* species based on trnH-psbA sequences.

Discussion

Avicennia genus with a few species but extensive geographical distribution may be an interesting case for styling the evolution and adaptive radiation. Adaptation to local environmental conditions which bring about extensive genetic changes as well as morphological differentiation, may lead to the evolution of distinct ecotypes or new species[1].

Avicennia species are known to show geographical and ecological preferences, as some of these species only grow in Atlantic-East Pacific (AEP), and some others are distributed in Indo-West Pacific (IWP). Some species like *A. marina* shows a wider geographical distribution ranging from Australia, to China, and middle east region. Therefore, investigating the evolutionary pathways and scenarios within this genus, may through light on local adaptations and ecological divergence within this genus.

The ecological divergence may occur surprisingly fast and even in absence of a physical barriers[1]. It has been suggested that the new beneficial mutations are unlikely to accumulate rapidly, and therefore, cases of fast adaptation may involve selection on standing genetic variation, i.e. genetic variation that was present in the ancestral population before divergence took place[17,18].

The present study revealed that some of the SNPs within genetic regions show geographical association and therefore have been selected for adaptation. These SNPs are from both nuclear ITS gene as well as chloroplast psbA genetic region.

Molecular phylogenetic studies performed by Li et al.[1], in *Avicennia*, resulted in divergence time estimates of 1.8-6.0 MYA within the IWP clade. They also reported two divergence events within the A. marina complex which were estimated to have occurred at 2.7 and 1.8 MYA, respectively. In general, the present study has some agreement with the reports of Van Belleghem et al.[4]. The difference in some details of estimates may be due to the fact that they utilized a combined data set of many nuclear and chloroplast genes together, but we should that these genetic regions may portrait different evolutionary pictures and therefore, we estimated the time of divergence separately for nuclear and chloroplast genes.

We obtained different divergence time for total sequence data in both ITS and psbA genetic regions, compared to that of adaptive sequences identified by LFMM. According to Van Belleghem et al.[4], It is possible to differentiate the alternative scenarios that describe the origin of standing genetic variation that describe the
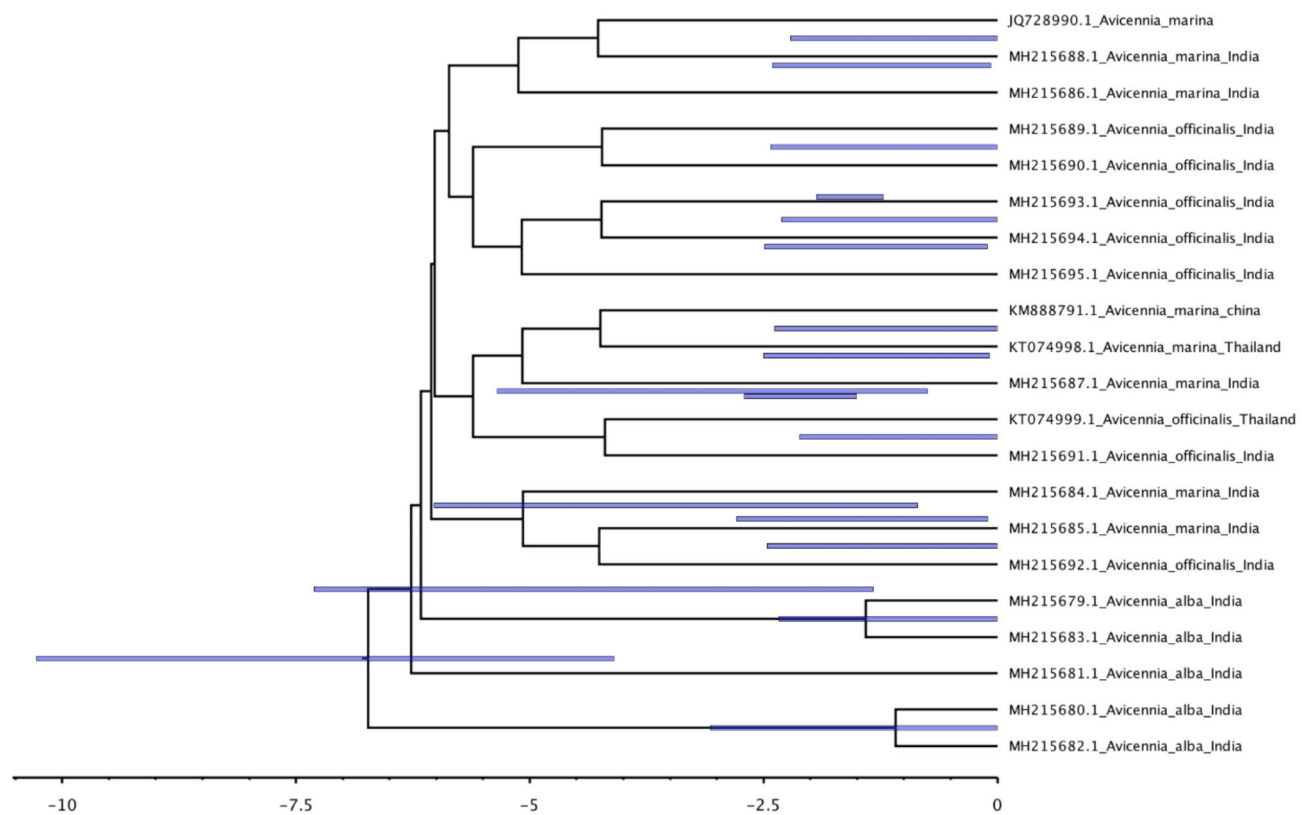
Fig. 4. BEAST chronological tree of *Avicennia* species based on trnL-F sequences.
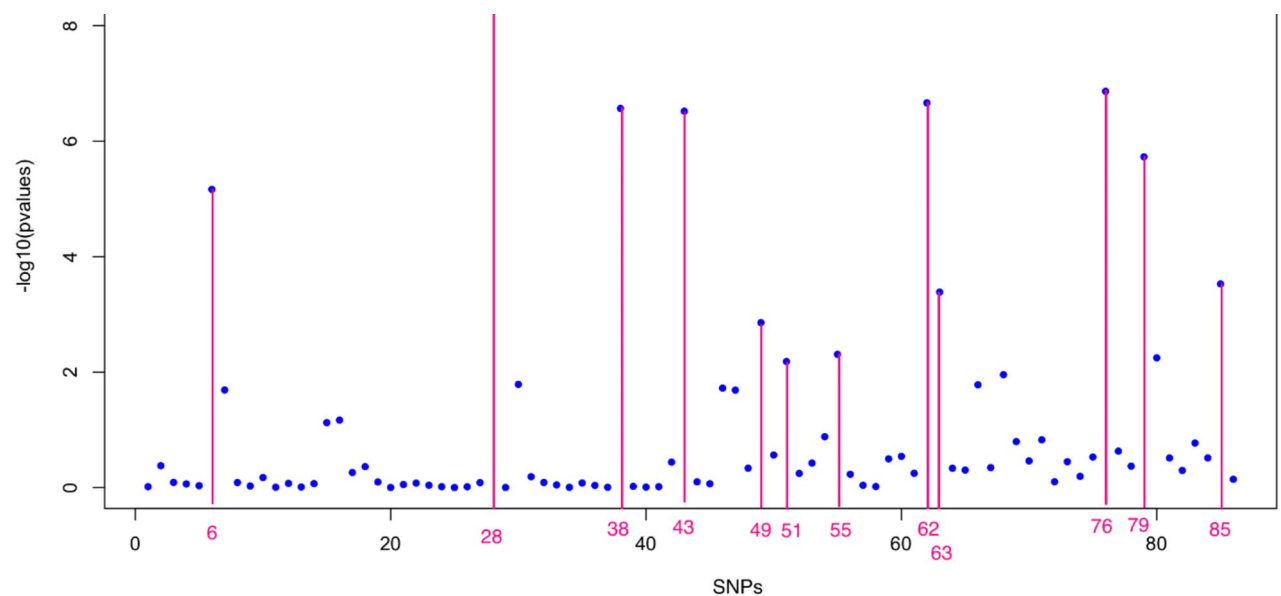
Fig. 5. Manhattan plot of trnH- psbA sequences (The SNPs showing significant association (log p-value > 2) are indicated with their SNP number).

demographic history of the populations or species. They suggest that if alleles involved in adaptation evolve through independent mutations, they occur at different loci or at random along the genealogy within a single locus. Therefore, they will not be identical-by-descent, because adaptive de novo mutations can occur on different haplotypes in different geographic regions. This may be the case for those SNPs within ITS and psbA regions of *Avicennia* which did not show strong phylogenetic signal.
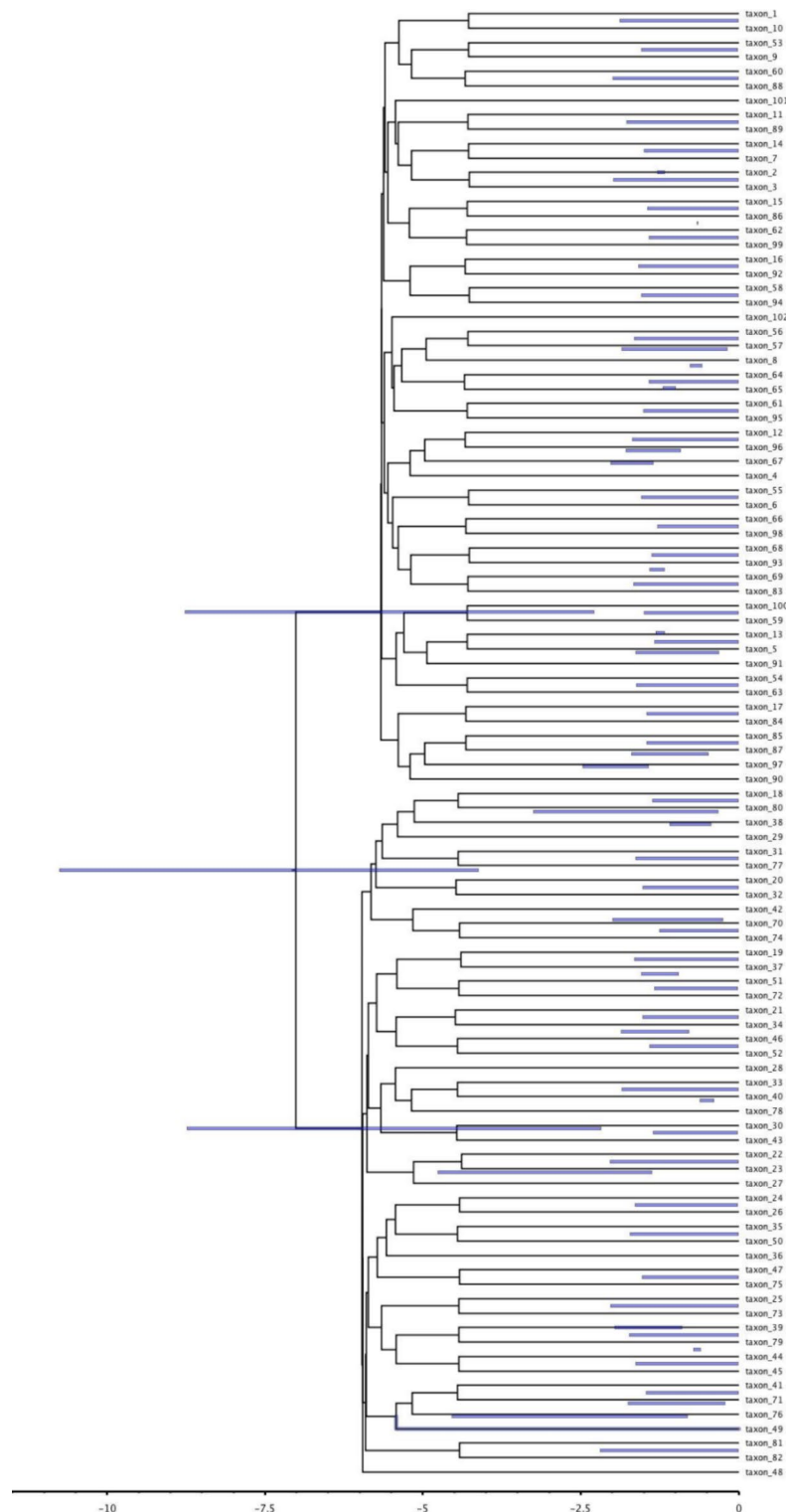
Fig. 6. BEAST chronological tree of adaptive ITS sequences.

Alternatively, if ecological differentiation is based on alleles that are present as standing genetic variation in the ancestral population, the derived alleles are expected to be identical-by-descent, but their evolutionary history may differ strongly at unlinked selected loci. This may be the case for SNPs with high phylogenetic signal within *Avicennia* genus. Therefore, we may have a complex selection and adaptive strategies in *Avicennia* genus, as both independent mutations as well. as local adaptations may have played role in the evolution of this genus.

The presence of heterogenous environmental conditions are known to cause changes in genetic diversity of plant species and result in local adaptations even in the populations of a single species[19]. This is one of the main
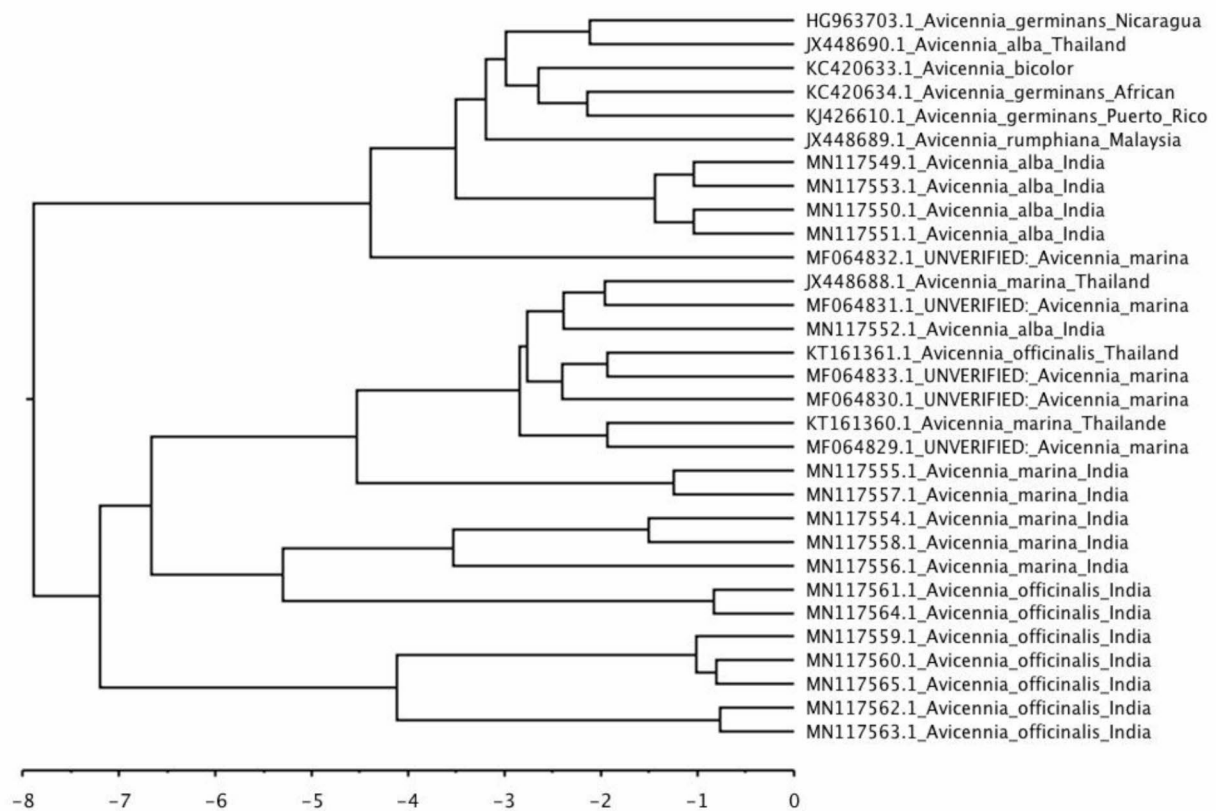
Fig. 7. BEAST chronological tree of *Avicennia* species based on merely adaptive trnH- psbA sequences.

reasons for genetic diversification within the genus *Avicennia*. Recent studies which are concerned with genetics of local adaptations try to answer two major questions: 1-which environmental variables play key role in the adaptive genetic divergence of a species or different species within a particular genus and shape its landscape genetic structure, and, 2-which genes or loci on the genome undergo adaptive genetic differentiation[20,21].In general, populations' local adaptation which leads to speciation within a genus is the act of natural selection in oppose to continuous gene flow.

In conclusion, the present study provide data on different DNA sequences changes in association with geographical variables. Moreover, we also suggest utilizing an analytical approach for estimating the time of nucleotide adaptations through divergence time analysis.
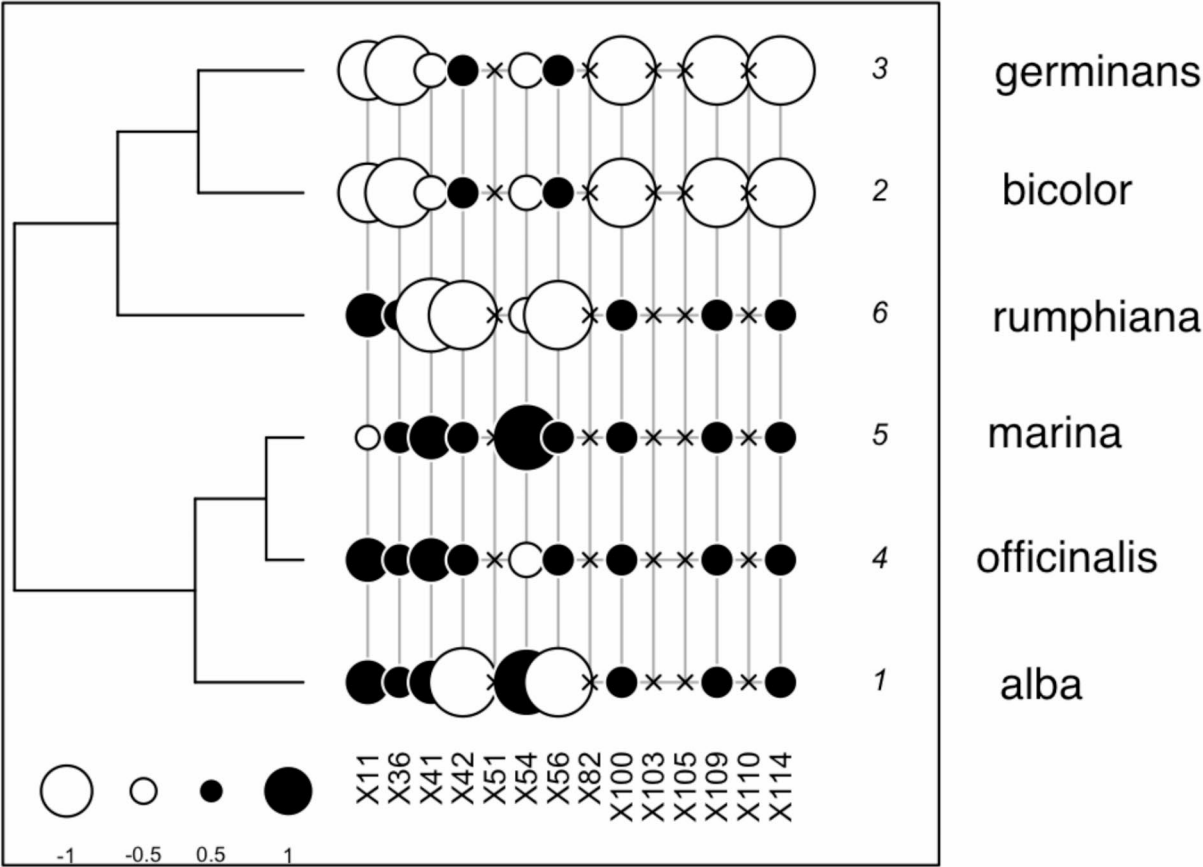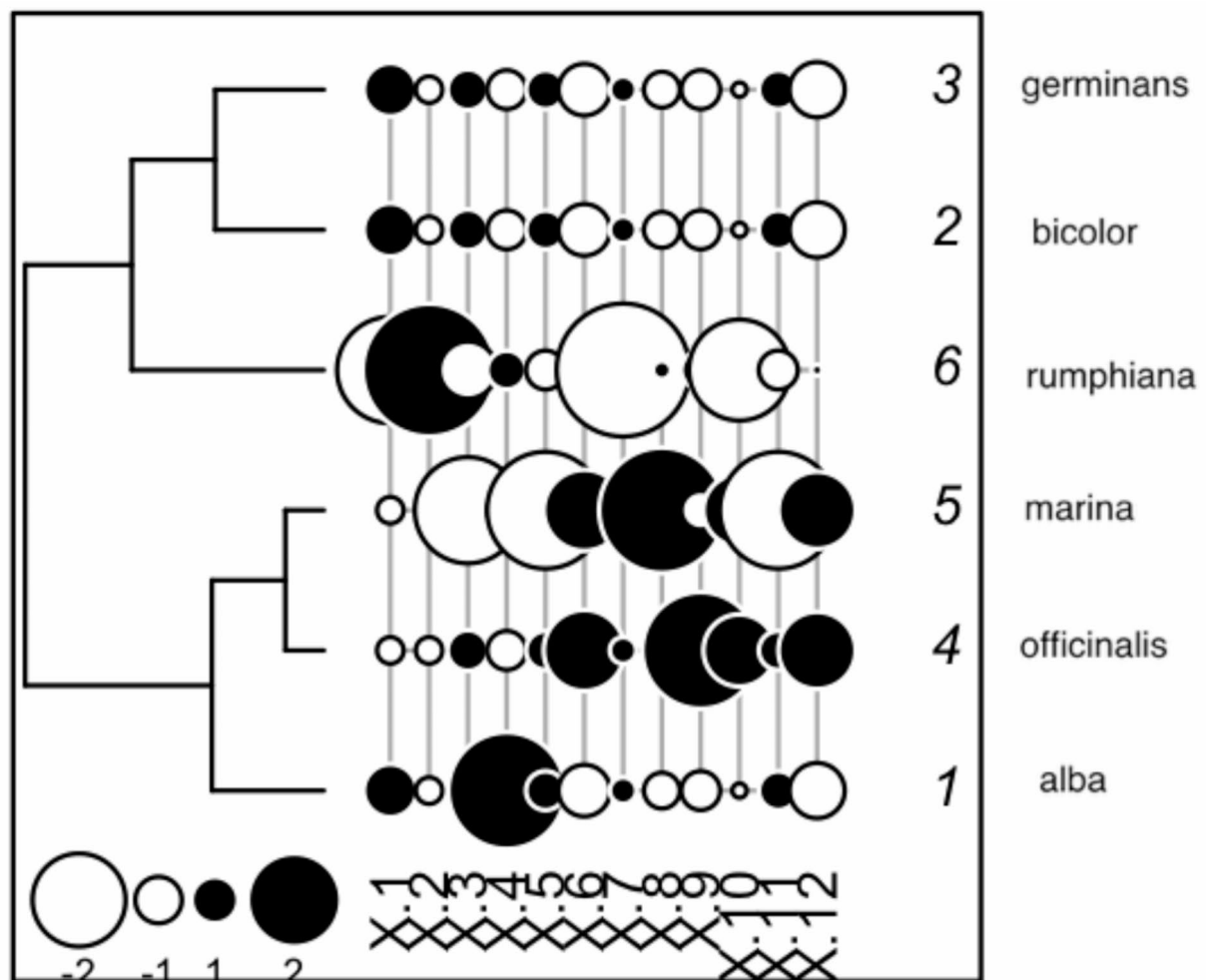
Fig. 8. Phylogenetic PCA of ITS sequences.

Fig. 9. Phylogenetic PCA of *Avicennia* species based on trnH-psbA sequences.

Data availability

The datasets analysed during the current study are available in the [National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/) repository], (Tables 1 and 2, and 3).

References

1. Li, X. et al. Re-evaluation of phylogenetic relationships among species of the Mangrove Genus *Avicennia* from Indo-West Pacific based on Multilocus analyses. *PLoS ONE*. **11**(10), e0164453 (2016).
2. Hrudayanath, T., Dibyajyoti, S. & Swagat, K. D. The genus *Avicennia*, a pioneer group of dominant mangrove plant species with potential medicinal values. *Front. Life Sci.* **9**, 267–291 (2016).
3. Duke, N. C. A systematic revision of the mangrove genus *Avicennia* (Avicenniaceae) in Australasia. *Aust Syst. Bot.* **4**(2), 299–324 (1991).
4. Van Belleghem, S. M. et al. Evolution at two time frames: polymorphisms from an ancient singular divergence event fuel contemporary parallel evolution. *PLoS Genet.* **14**(11), e1007796 (2018).
5. Tamura, K., Stecher, G. & Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **38**(7), 3022–3027 (2021).
6. Bouckaert, R. et al. BEAST a Software platform for bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**(4), e1003537 (2014).
7. Nee, S. Birth-death models in macroevolution. *Annu. Rev. Ecol. Evol. Syst.* **37**, 1–17 (2006).
8. Drummond, A. J., Rambaut, A. & Xie, W. Apr. BEAUti version 1.6.1. 2012a. http://ww2.beast.bio.ed.ac.uk/, Accessed 20 Apr 2011.
9. Drummond, A. J. & Rambaut, A. BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214 (2007).
10. Rambaut, A. Fig Tree v1.3.1: tree figure drawing tool (2009). http://ww2.tree.bio.ed.ac.uk/software/figtree/
11. Legendre, P. & Legendre, L. Numerical ecology, 3th edn. Oxford, UK (2012).
12. Hammer, Ø. & Harper, D. A. Past: Paleontological statistics software package for educaton and data anlysis. *Palaeontologia Electron*. **4**(1), 1 (2001).
13. Frichot, E. & Francois, O. LEA: An R package for landscape and ecological association studies. In press.
14. Frichot, E., Schoville, S. D., Bouchard, G. & Francois, O. Testing for associations between loci and environmental gradients using latent factor mixed models. *Mol. Biol. Evol.* **30**(7), 1687–1699 (2013).
15. Revell, L. J. Size-correction and principal components for interspecific comparative studies. *Evolution*. **63**, 3258–3268 (2009).
16. Jombart, T., Pavoine, S., Devillard, S. & Pontier, D. Putting phylogenyinto the analysis of biological traits: Amethodological approach. *J. Theor. Biol.* **264**, 693–701 (2010).
17. Zou, X. H. et al. Analysis of 142 genes resolves the rapid diversification of the rice genus. *Genome Biol.*; **9**(3). (2008).
18. Mori, G. M., Zucchi, M. I., Sampaio, I. & Souza, A. P. Species distribution and introgressive hybridization of two *Avicennia* species from the Western Hemisphere unveiled by phylogeographic patterns. *BMC Evol. Biol.* **15**, 61 (2015).
19. Segovia, N. I., González–Wevar, C. A. & Haye, P. A. Signatures of local adaptation in the spatial genetic structure of the ascidian Pyura chilensis along the Southeast Pacific coast. *Sci. Rep.* **10**, 14098 (2020).
20. Li, Y. et al. Ten years of landscape genomics: Challenges and opportunities. *Front. Plant. Sci.* **8**, 2136 (2017).
21. Zhang, X. X. et al. Landscape genetics reveals that adaptive genetic divergence in *Pinus bungeana* (Pinaceae) is driven by environmental variables relating to ecological habitats. *BMC Evol. Biol.* **19**, 160 (2019).

Author contributions

M. Sh, conceptualized and designed the present project. L.M. collected and analyzed parts of the data. F.Gh. and A.D. and F.K. analyzed parts of the data. The first draft of the manuscript was written by M. Sh. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Additional information

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