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Avicennia genus molecular phylogeny and barcoding: A multiple approach

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Abstract. The genus *Avicennia* contains of 8 species which show a great extent of morphological and genetic variability, which make taxonomy of the genus difficult. Molecular barcoding along with advancement in computational approaches may be proper methods to investigate and assess the efficiency of different molecular genetic regions in *Avicennia* species delineation and also produce data on species evolution and divergence. The aims of present study were to utilize multiple genetic data for the species delineation and study the phylogeny of the genus. Moreover, we developed a hypothesis on biogeography of these species with respect to barcode divergence. The results showed that both Internal transcribed spacer (ITS) and trnHG–psbA intergenic spacer (trnHG-psbA) sequences may be used in *Avicennia* species delineation. Barcode gap analysis and nucleotide difference of the studied taxa showed significant Fst for pairwise species comparison and the role of nucleotide changes in *Avicennia* speciation.

Keywords: *Avicennia*, Barcode-gap analysis, Genetic differentiation, Nucleotide difference speciation.

INTRODUCTION

DNA barcoding is applied to plant and animal species with the aim to improve organismal identification and taxonomic clarification. The main principles of DNA barcoding are standardization, minimalism, and scalability, which means selection one or a few standard loci that can be sequenced routinely and reliably in very large and diverse sample sets, and obtaining a reliable and conveniently comparable data to differentiate the species in question from one another (Hollingsworth et al. 2011).

Controversy exits on the use and choosing the plant molecular barcode markers. Different researches resulted in general agreement that several different marker combinations produce equivalent performance, and that none of the proposed barcodes is perfect in every respect (Seberg and Petersen

2009). Utilizing a multiple approach for a better species differentiation has been suggested by several authors (see for example, Fazekas et al. 2008).

In most of the studies, researchers use of a common, easily amplified and aligned region such as *rbcL*, *trn L-F* spacer regions, *mat K*, *trnHG-psbA*, *nrITS1*, *nrITS2* or the full *ITS1-5.8S-ITS2* (*nrITS*), as suggested by the CBOL Plant Working Group and BOLD (Cbol 2009; Ratnasingham and Hebert 2007).

The genus *Avicennia* is composed of eight species of mangrove trees which grow in intertidal zones in tropical and temperate regions of the world. These plant species are economically important as they are extensively used as medicinal plants. In fact, different parts of these plants have ethno medicinal applications for treatment of various diseases such as cancer, diabetes, malaria, rheumatism, asthma, small pox and ulcer (Hrudayanath et al. 2016). These species show variation and are taxonomically complex due to vast geographical distribution and introgressive hybridization (Mori et al. 2015). Therefore, the aims of present study are: 1- Assessment of different molecular markers in *Avicennia* species delineation through barcode analysis, 2- Species relationships based on molecular markers, and 3- Biogeographical distribution of these species with regard to DNA sequence divergence.

Table 1. Voucher information and GenBank accession numbers of taxa sampled for the genus *Avicennia* based on trnL-F 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

MATERIALS AND METHODS

In this study, we used published data on *trn L-F*, *trnHG-psbA* and *ITS* sequences for a number of *Avicennia* species which are reported from different parts of the world in NCBI site (Table 1, 2 and 3).

Data analyses

DNA sequences obtained were initially aligned by MUSCLE program and cured accordingly. The total length, polymorphic sites, average of p distance, Genetic diversity within the studied species, the Fst values for the sequences and Maximum Liklihod phylogenetic tree based on these sequences as well as Tajimas'D test was performed as implemented in MEGA ver. 7 (Kumar et al. 2016). Mantel test performed with 1000 permutations, for showing significant association between nucleotide difference of the studied species and population to the geographical longitude and altitude. The range of Bayesian probability value obtained for species with Mr. Bayes analysis (Ronquist et al. 2012). Barcoding analysis and windows sliding of nucleotides were performed by BarcodingR, and spider package, while Skyline plot and

mismatch distribution of nucleotides were determined by ape, and pegas package in R. Monophyletic phylogenetic tree and its statistical test of Rosenberg (2007), as implemented in R package. We used RASP (Reconstruction of ancestral states in phylogeny) program ver. 4.2 in the RASP-Bayesian analyzed phylogenetic tree.

RESULTS

Species delimitation and barcode gap analysis

We used published data on *trn L-F*, *trnHG-psbA* and *ITS* sequences for a number of *Avicennia* species which are reported from different parts of the world (Table 1). In the first step, we evaluated the efficiency of these molecular markers in species delineation, and finally, we extracted different evolutionary information from the one with the highest degree of efficiency.

Details of the studied sequences with regard to *Avicennia* species differentiation and species phylogeny are presented below. Available data on *trn L-F* sequence is very limited and is available for only 21 samples of *Avicennia officinalis*, *A. marina*, and *A. alba*. These sequences had a total length 296 bp, with only 14 polymorphic sites, and average p distacne = 0.006. The ML phyloge-

Table2 . Voucher information and GenBank accession numbers of taxa sampled for the genus *Avicennia* based on trnHG-psbA data.
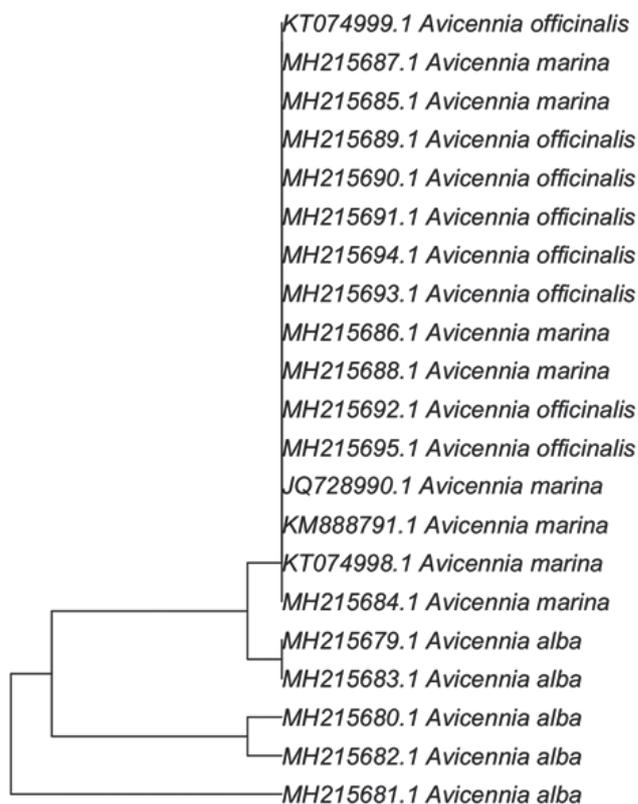
Sp	Accession number
Avicennia officinalis	KT161361.1
Avicennia officinalis	MN117565.1
Avicennia officinalis	MN117564.1
Avicennia officinalis	MN117563.1
Avicennia officinalis	MN117562.1
Avicennia officinalis	MN117561.1
Avicennia officinalis	MN117560.1
Avicennia officinalis	MN117559.1
Avicennia alba	MN117553.1
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 germinans	KC420634.1
Avicennia germinans	KJ426610.1
Avicennia germinans	HG963703.1
Avicennia bicolor	KC420633.1
Avicennia rumphiana	JX448689.1

Figure 1. ML Phylogenetic tree of *Avicennia* species based on ITS sequences.

netic tree based on these sequences (Fig. 1), revealed that only the samples of *A. alba* can be differentiated from the other two species.
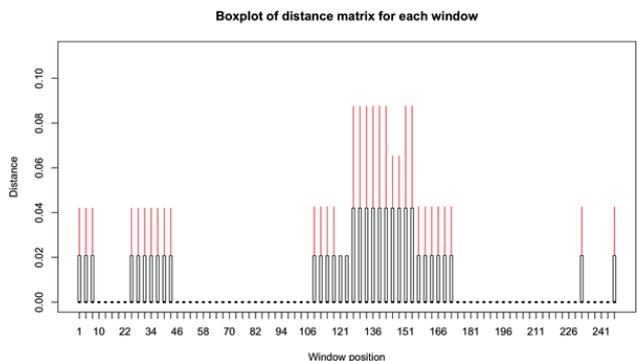
Analyses performed by Bayesian method of species barcoding as implemented in Barcoding package in R program indicated that only 25% of the studied samples have success in species identification, and the others may not be differentiated. In this analysis also the higher degree of Bayesian probability was obtained for *A. alba* (0.50-0.97). The probability value obtained for the other species was about 0.06 only. Sliding Windows of the mini-barcodes within *trn L-F* barcode sequence 9 (Fig. 2), also showed genetic distance in mini-barcodes between *A. alba* and the other species studied.

TrnHG-psbA sequence efficiency in species delineation and barcoding

TrnHG- psbA sequence data is available for 26 sample in *Avicennia* species (Table 2) namely, *A. officinalis,*

Figure 2. Mini-barcode sliding windows of *trnL-F* sequence in *Avicennia* species, showing genetic distance of *Avicennia alba* with the other studied taxa.

A. marina, A. bicolor, A. germinans, A. alba, and *A. rumphiana.* These samples have also been reported from different parts of the world.

Preliminary analysis of *trnHG- psbA* sequences indicated that the total length of the studied sequences is 167 bp, with 52 polymorphic sites, and average p distance = 0.09.

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

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

| Sp	Accession number
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
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

Sp	Accession number
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
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

Bayesian method analysis of barcoding for *trnHG-psbA* sequences, reveals that about 53% of the samples are identified with success. Bayesian probability value obtained for *A. officinalis* samples, ranged from 0.24 to 0.96. The same value for *A. germinans*, ranged from 0.5-0.95, for *A. marina* and *A. alba* the value ranged from was 0.2 to 0.96.

Monophyletic phylogenetic tree and its statistical test of Rosenberg (2007), as implemented in R package is presented in Fig. 3. The red circles on the nodes of this phylogenetic tree indicates that monophyly has passed the significant test at p = 0.05. Therefore, we observe the
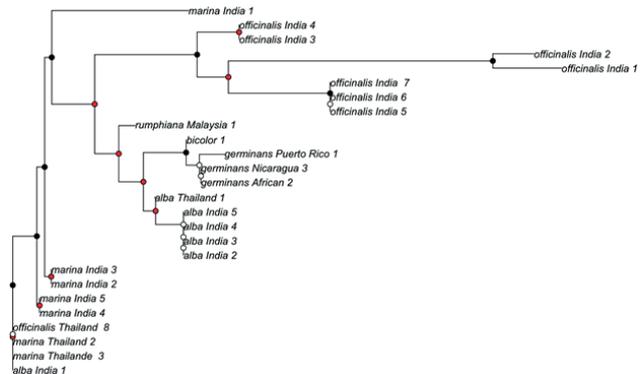
Figure 3. Monophyly analysis of *Avicennia* species based on *trn-HG-psbA* sequences. [a]The red circles on the nodes indicate that the clade is significant at p = 0.05.

Nucleotide Difference
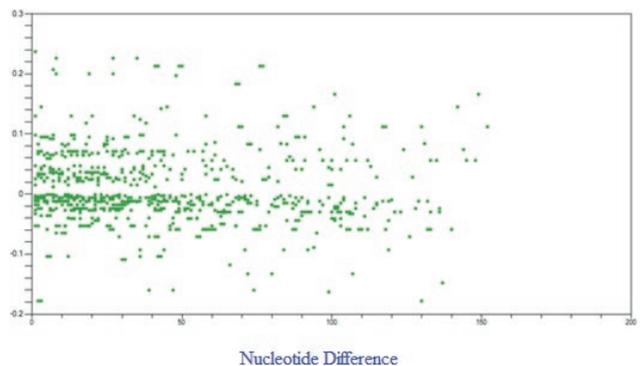
Figure 4. *trnHG-psbA* nucleotide difference among *Avicennia* species.

presence of significant monophyletic groups for some of the sample in *A. officinalis*, *A. alba*, *A. germinans*, and *A. marina*. We have also some cases of admixture between the studied species which makes that clade non-monophyletic.

TrnHG- psbA nucleotide difference in the studied *Avicennia* species is presented in Fig. 4. This plot shows a great difference of the *trnHG- psbA* nucleotides, which is a significant difference according to chi-square and Snn test of Hudson (Hudson, 2000).

The Fst values for *trnHG- psbA* sequences in the studied species ranged from 0.40-0.65. Inter-specific genetic differentiation estimates obtained for the *trnHG- psbA* nucleotide produced chi-square = 63.321, with P-value = 0.0012. Similarly, Hudson Snn after 1000 permutations produced Snn = 0.86, P<0.01. These values indicate significant difference among the studied samples.

Pair-wise mismatch plot of these sequences (Fig. 5), revealed that, almost all the studied species-pairs differ significantly in their *trnHG- psbA* nucleotides.
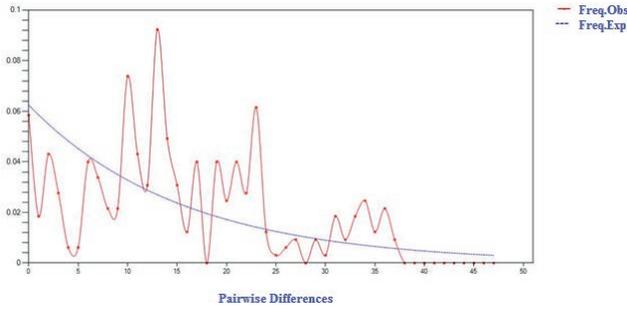
Figure 5. Pair-wise mismatch plot of *trnHG-psbA* sequences in *Avicennia* species.
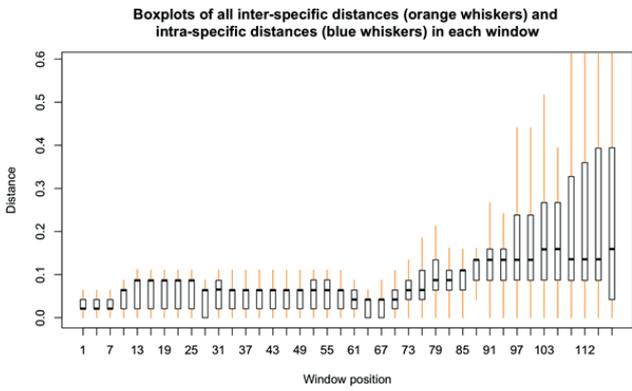

Figure 6. Window sliding of *trnHG-psbA* sequences in *Avicennia* species, showing that these species differ in mini-barcodes.

Mini-barcode analysis by windows sliding (Fig. 6), also showed that *trnHG- psbA* sequences contain mini-barcodes which differs among *Avicennia* species. Therefore, *trnHG- psbA* sequences could be utilized for *Avicennia*. species delineation.

Mini-barcode analysis by windows sliding (Fig. 7), also showed that *trnHG- psbA* sequences contain mini-barcodes which differs among *Avicennia* species. Therefore, *trnHG- psbA* sequences could be utilized for *Avicennia* species delineation.

ITS sequences species delineation and barcoding

ITS sequences (Table3) obtained had total length 494 bp, with polymorphic sites = 88, and average p dist = 0.038. Bayesian method analysis revealed that about 42% of the samples are identified correctly. Bayesian probability value obtained for *Avicennia alba* ranged from 0.12 to 0.99, for *A. bicolor* ranged from 0.20 to 0.99, and for *A. germinans*, ranged from 0.16 to 1.00. Almost the same ranges were obtained for other species. ML phylogenetic tree of 135 species samples (Fig. 8), pro-

Figure 7. *TrnHG-psbA* sequences in *Avicennia* species, showing mini-barcodes which differentiate the studied taxa.
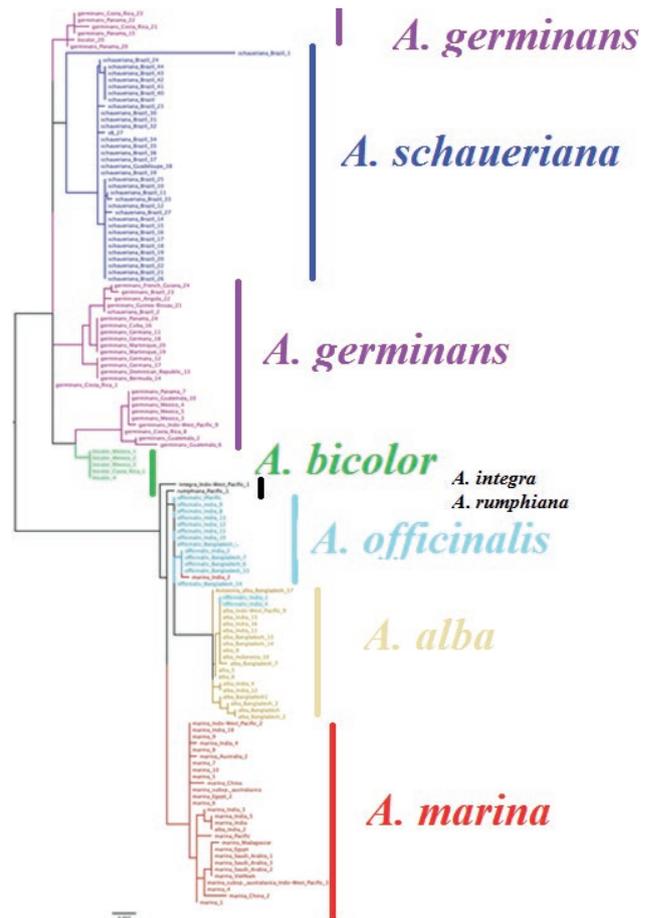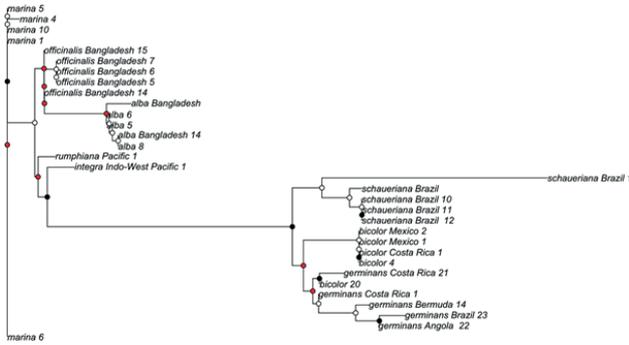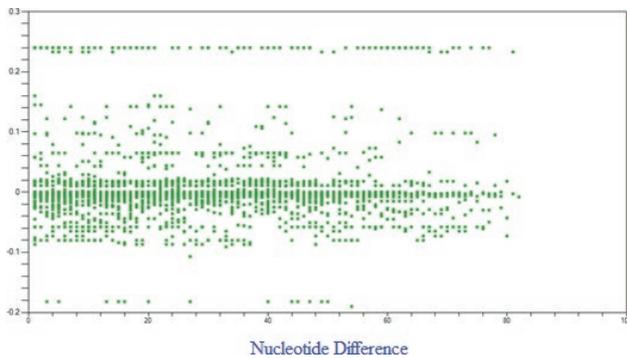

Figure 8. ML Phylogenetic tree of *Avicennia* species based on ITS sequences.

Figure 9. Monophyly test of the studied *Avicennia* species based on ITS sequences. [a]The red circles on the. nodes indicate a significant monophyletic clade.
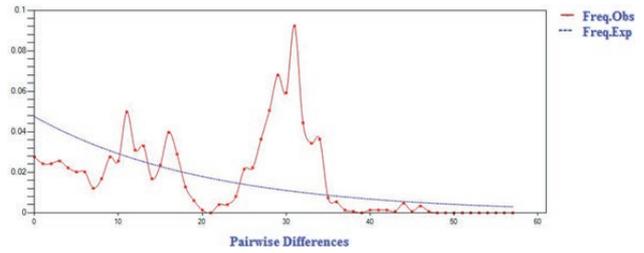

Figure 10. The nucleotide difference in ITS sequences among *Avicennia* species.

duced almost distinct separate clades for the studied species, but some degree of admixtures was observed too. For the test of monophyly, we kept randomly some of the replicates of each species. The result of monophyly and its statistical test of Rosenberg is presented in Fig. 9. The red circles on some of the tree nodes indicates the monophyletic clade which is significant at p = 0.05.

We also obtained monophyletic clades for some of the sample in *A. officinalis*, *A. alba*, *A. germinans*, *A. bicolor* and *A. marina*. We have also some cases of admixture between the studied species which makes some of the clade non-monophyletic.

The nucleotide difference in ITS sequences of the studied *Avicennia* species is presented in Fig. 10. The plot shows a great difference, which is a significant difference according to chi-square and Snn test of Hudson.

The Fst values for ITS sequences ranged from 0.43-0.99. The inter-specific genetic differentiation estimates obtained for the ITS nucleotide produced chi-square = 90.26, with P-value = 0.001. Similarly, Hudson Snn after 1000 permutations produced Snn = 0.82, P<0.001. These

Figure 11. Mismatch plot of ITS sequences in *Avicennia* species.

Figure 12. ITS Barcode sequences differentiating *Avicennia alba* and *A. bicolor*.

values indicate significant difference among the studied samples. Mismatch plot of ITS sequences (Fig. 11), revealed that, almost all the studied species-pairs differ significantly in their *trnHG- psbA* nucleotides. Moreover, Tajimas'D obtained was 0.32, which indicates the presence of a positive selection on ITS sequences and therefore ITS changes may be in some way related to speciation events in the genus *Avicennia*.

Windows sliding of ITS sequences also revealed the occurrence of mini-barcodes in these sequences which also differed greatly among the studied species. For example, barcode sequences in some of the species are provided in Figs 12 and 13.

Genetic diversity within the studied species ranged from 0.03 in *A. alba* to 0.12 in *A. germinans*, while interspecific genetic distance, 0.04 between *A. alba* and *A. officinalis*, and 0.08 between *A. marina* and *A. integra*, to 0.43, between *A. germinans* and *A. alba*.

DNA barcoding gap analysis of ITS sequences is presented in Fig. 14. Both intra- and inter-specific sequence gaps, supports the use of ITS sequences for delineation of *Avicennia* species.

Mantel test performed with 1000 permutations, showed no significant association between nucleotide difference of the studied species and population to the geographical longitude and altitude (Correlation r = 0.044, p = 0.1456).

Details of *Avicennia* species diversification based on ITS sequences and in relation with geographical distri-

Figure 13. Barcode ITS sequences differentiating *Avicennia alba* and *A. germinans*.
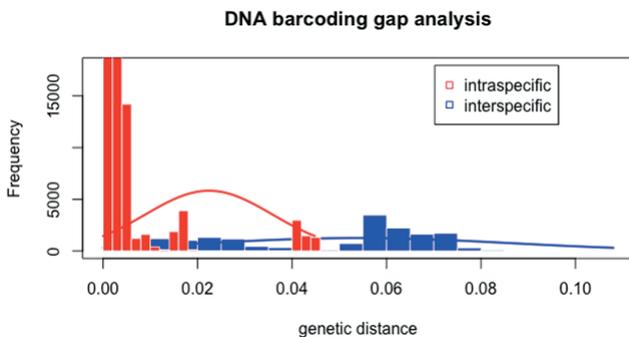
Figure 14. Barcode gap analysis of ITS sequences revealing both intra- as well as inter-specific sequence difference in *Avicennia* species studied.

bution of these species is presented in the RASP-Bayesian analyzed phylogenetic tree (Fig. 15).

Two main clades are present in this phylogenetic tree. The species of *A. alba*, *A. officinalis*, *A. marina*, *A. rumphiana*, and *A. integra*, comprised the first major clade, while, *A. schueriana*, *A. germinnas*, and *A. bicolor* formed the second major clade.

Looking at details of each major clade points out some interesting results. For example, most of *A. marina* samples were grouped together due to sequence similarity. Though Mantel test revealed no association between nucleotide difference and geographical longitude and latitude of the studied taxa, some interesting relationships between *A. marina* geographical populations can be seen
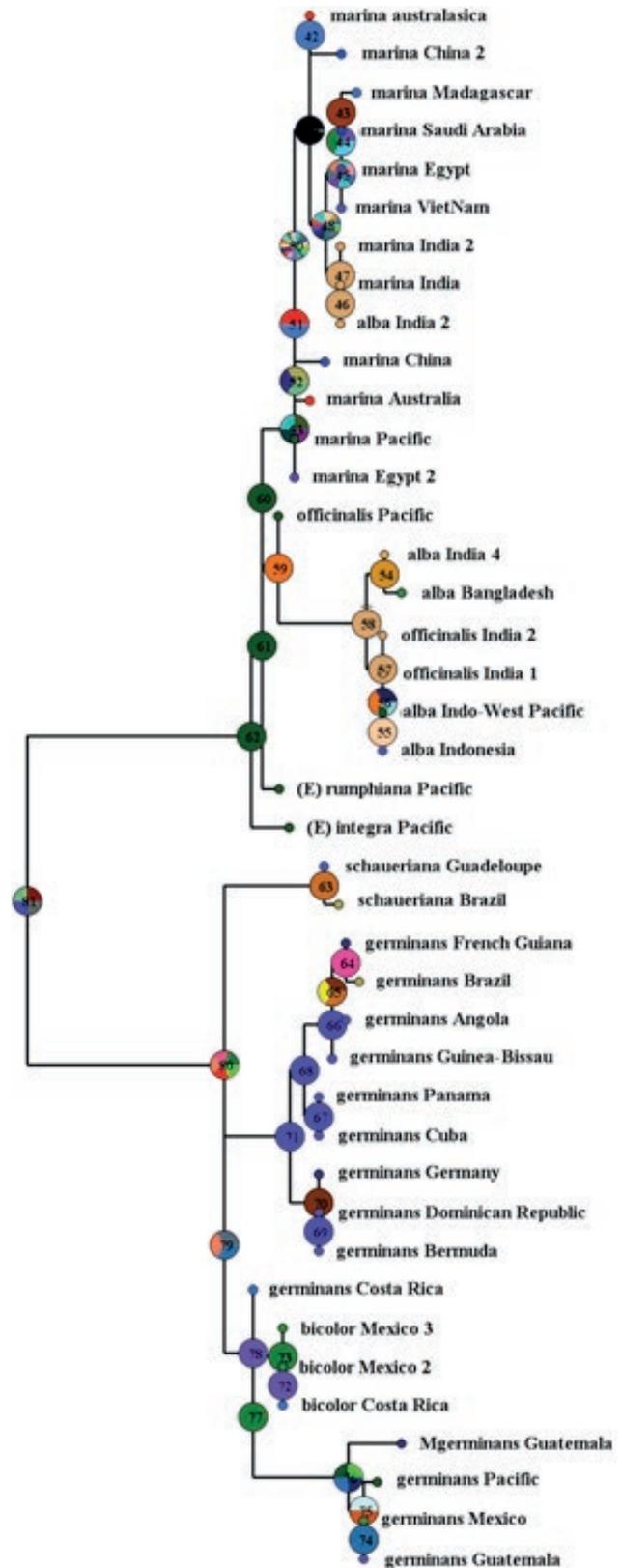
Figure 15. RASP Bayesian tree of ITS data, placing the species studied in two major clades.
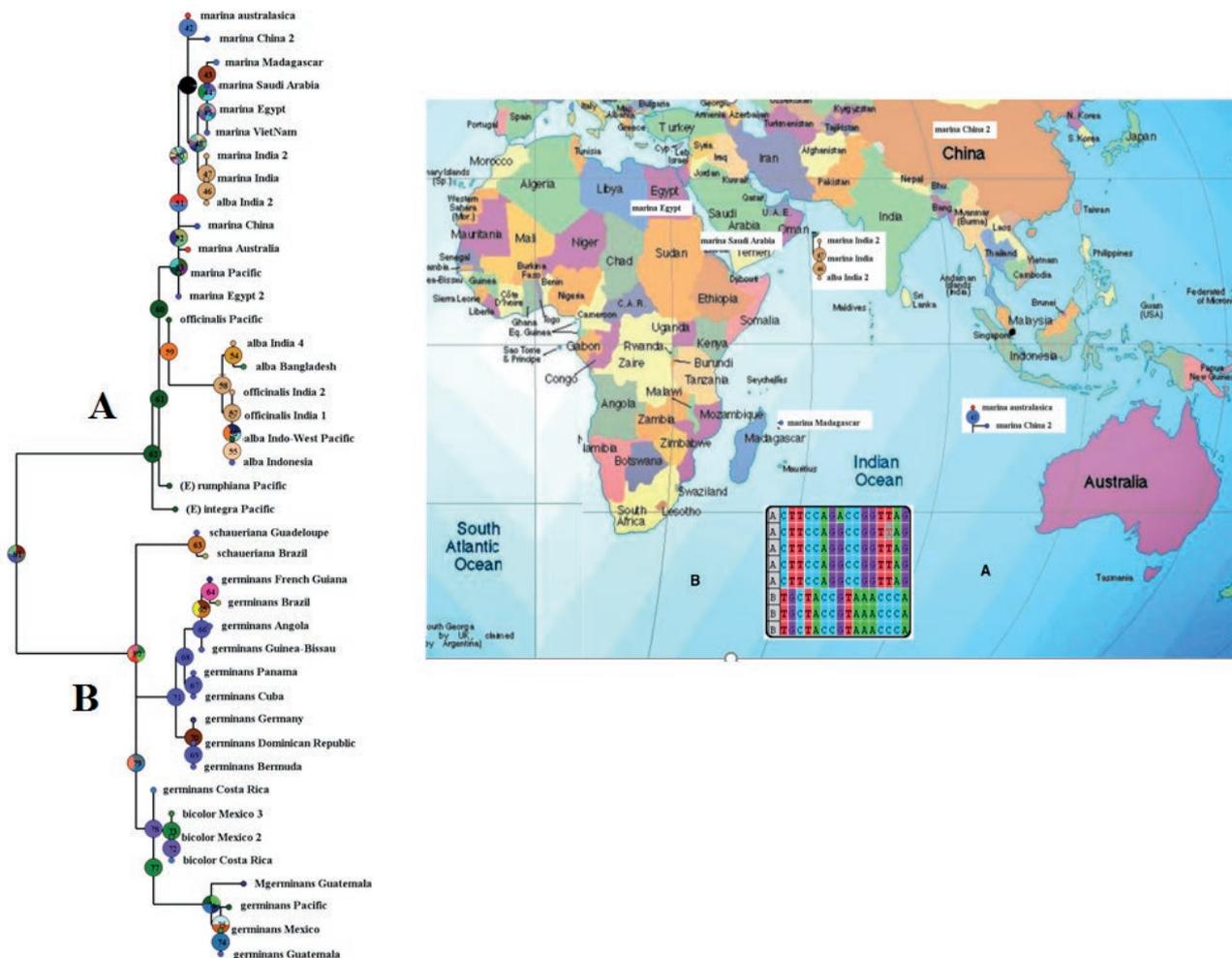
Figure 16. Biogeographical distribution of *Avicennia* marina populations based on ITS sequences.

when we plot these studied specimens on the world map. For example, *A. marina* specimens studied from Saudi-Arabia and Egypt show sequence affinity and are placed close to each other in the phylogenetic tree. Moreover, the specimens studied from Madagascar, is placed close to the above said specimens. Madagascar is some-what close to and connected by Indian ocean to Saudi-Arabia and Egypt.

Similarly, *Avicennia marina* samples from China and Australia, also show sequence similarity and form a separate sub-clade from the other specimens studied. The studied specimens from India stand in a separate sub-clade, far from the sub-clade of China-Australia.

If we consider all the samples studied, biogeographical distribution reveals that the species of *A. marina*, *A. alba*, and *A. officinalis* are mostly found in Asia and Australia region (Denoted A. in Fig. 16), while *A. terminals*, *A. bicolor*, and *A. schauriena*, are distributed in South America (Denoted B in Fig. 16). This may indicate speciation events in *Avicennia* in two different regions of the world. We have also provided barcodes for geographical regions A and B, which shows nucleotide changes possibly associated / or the outcome of speciation in these two areas (Fig 16).

DISCUSSION

The present study showed that based on ITS sequence analysis, *A. alba* and *A. officinalis* show close affinity and comprise sister-clades. *A. marina* joins these two with some distance.

A. germinans samples form three separate clades, which indicates a potential presence of infra-specific taxon rank within this species. This is in accordance with earlier consideration which propose three different vari-

eties for this species, which were later on were merged into a single species with no variety.

A. bicolor showed close relationship to one of the clades of A. germinans. Sample of A. schaueriana comprise a single distinct clade based on ITS data.

However, the species relationships were partly distorted by trnHG- psbA sequence data. The three species of A. officinalis, A. marina, and A. alba, were placed inter-mixed to some degree. Close affinity bet ween A. germinans and A. bicolor are similar to ITS results. The close affinity between A. officinalis, A. marina, was also indicated by Li et al. (2016). These authors showed closer relationship between A. rumphiana and A. alba, which in agreement with our trnHG- psbA tree, and to some extent also with ITS-baes phylogenetic tree.

According to Li et al. (2016), even though the first fossil record of Avicennia in the IWP region dates back to the late Eocene of southwest Australia, Avicennia speciation was active during the Miocene. Similarly, they suggest that distribution of ancestral Avicennia was likely to have been similar to its present location, extending from Japan to Borneo and from the Marshall Islands to the Red Sea. Therefore, the materials studied in this project, may have migrated from Japan, china or India, through red sea and reached to the countries like Iran, Egypt, and Sauidi-Arabia. In these migration path, Avicennia speciation may have resulted in formation of A. marina, A. officinalis and A. alba, as well as A. integra, and A. rumpiana.

If we consider our ITS-based phylogenetic tree, we observe the species of the region B, viz. A. bicolor, A. germinans, A. schaueriana. are related through A. rumpiana and A. integra, to the species of region A. Therefore, we may suggest a preliminary hypothesis that through migration of either or both of A. rumpiana and A. integra, new speciation events resulted in the formation of other species found in South America countries. We believe that more works are required to second this raw and immature hypothesis.

Mangroves in general, have a broad distributional patterns, ability in long-distance dispersal and can adapt to rigorous environmental constraints associated with regular seawater inundation. However, the present day distributions of individual taxa show several instances of finite dispersal limitations, especially across open water. These dis-continuities, in the absence of current dispersal barriers, may be explained by persistent past barriers (Duke et al. 2002).

In present study we report genetic diversity both with the studied Avicennia species and between these taxa. A high levels of genetic diversity were also reported among the central populations of many mangrove species including Avicennia in the Indo-West Pacific (IWP) (Mantiquilla et al. 2021). Mori et al. (2015), suggest that A. bicolor, A. germinans and A. schaueriana are three evolutionary lineages that present historical and ongoing hybridization. They also consider gene flow between A. germinans, and A. schaueriana by propagules rather than pollen in A. schaueriana.

We also reported distinct inter-specific genetic distance and significant Fst value among different species both within those species distributed in the A geographical region (Australia, India, China), and those distributed in the B region (South America in general). In a similar investigation performed presence of a strong genetic structuring resulting in divergence among mangrove populations of Indian Ocean and South China Sea, as well as between South China Sea and Southwestern Pacific was reported (Mantiquilla et al. 2021).

CONCLUSION

With regard to Avicennia species taxonomy and the presence of high level of genetic diversity within these species, we provided distinct molecular barcodes for species delineation. We suggest it is suitable to utilize a combination of ITS nuclear sequences along with trnHG- psbA spacer region of chloroplast genome for taxonomic purpose.

LIST OF ABBREVIATIONS

ITS: Internal transcribed spacer
ML: Maximum Liklihod
RASP: Reconstruction of ancestral states in phylogeny
MEGA: Molecular Evolutionary Genetics Analysis

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AUTHOR CONTRIBUTION STATEMENT

Masood Sheidai: conceptualization of the project and corresponding author, Farrokh Ghahremaninejad: conceptualization of the project and data collection, Afshin Danehkar: conceptualization of the project and plant collection, Fahimeh Koohdar: conceptualization of the project and lab work, Laleh malekmohammadi: data collection and lab work and data analysis.

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