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DNA BARCODING OF THE TRUE MANGROVE PLANTS, A SELECTION OF GENETIC MARKERS

Nguyen Xuan Vy[1*], Nguyen Nhat Nhu Thuy[1], Nguyen Trung Hieu[1], Luong Quang Doc[2]

[1]*Department of Marine Botany, Institute of Oceanography, VAST*
[2]*Department of Biology, College of Sciences, Hue University*
[*]E-mail: nguyenxuanvi@gmail.com

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ABSTRACT: Classically, morphological classification has been considered the main approach. However, this method sometimes shows the difficulties due to requirement of the full morphological characters from the specimens. Additionally, the disadvantages of this method are that it does not resolve the closely related species which are overlapping in terms of morphological characters and high plasticity in some genera. DNA barcoding is an approach to identify species by using short orthologous DNA sequences, known as 'DNA barcodes' and requires a very small tissue. This study is based on 59 ITS sequences including 55 ITS sequences retrieved from the GenBank and 4 sequences gained from three species of *Lumnitzera* collected in Vietnam. The methods of Maximum Likelihood, Neighbor Joining, Maximum Parsimony and Bayesian Inference based on ITS were applied to construct the phylogenetic tree of mangrove. Our results showed that there was no difference between *Lumnitzera* x *rosea* and *L. littorea*, and the species resolution based on ITS was higher than the concatenated *rbc*L/*mat*K sequences which were suggested as DNA barcodes for the plant. In conclusion, ITS could be applied as a DNA barcode for mangroves instead of the *rbc*L/*mat*K system previously proposed.

Keywords: DNA barcodes, mangroves, ITS, *Lumnitzera*.

INTRODUCTION

Classically, the method based on morphological characters was a traditional technique to name the species. For the mangrove, the morphology of flowers, leaves, fruits is also used to identify the specimens [1]. However, their flower and fruit are seasonal. On the other hand, morphological variants within species as well as homomorphism among closely related species are very common in marine macrophytes. Additionally, the hybridisation among two species forming the new species is also common in mangroves [2], it is really challenge for the taxonomists. Hence, morphological identification may lead to the misidentification of taxa. To overcome the disadvantages of morphological identification, the new approach for species identification based on genetic markers or DNA barcoding has been widely used.

DNA barcoding is an approach to identify and recognize species by using short orthologous DNA sequences, known as 'DNA barcodes'. Today, the Cytochrome C oxidase (COI) was considered DNA barcode for animals [3]. The combination of two genes including Ribulose-1,5-bisphosphate-carboxylase-oxygenase (*rbc*L) and Chloroplast maturase K (*mat*K) was suggested as DNA barcodes for plants [4]. The species resolution by a combination of *rbc*L/*mat*K barcodes showed more advantages than previous markers

such as $tRNA^{Leu}$ UAA (*trn*L) [5], *mat*K [6] or concatenated *trn*L and *rbc*L [7]. However, *rbc*L/*mat*K did not resolve the species boundaries between two closely related species, for example, two seagrass species *Halophila ovalis* (R.Br.) Hook. and *Halophila major* (Zoll.) Miquel. [8]. Recently, Nguyen et al., [9] indicated that the internal transcribed spacer (ITS) including ITS1-5.8S-ITS2 showed higher species resolution than *rbc*L/*mat*K barcodes in the case of seagrasses. Hence, ITS was now suggested as DNA barcodes for seagrass [10].

Family Combretaceae includes 18 genera, and three of them are mangroves. Among three genera, three species of *Lumnitzera* were found in Vietnam. *Lumnitzera racemosa* Willd. is very common species while *L. littorea* (Jack) Voigt is very rare [11]. Tomlinson [12] found that *Lumnitzera* x *rosea* (Gaudich.) C. Presl was the hybrid of *Lumnitzera racemosa* and *L. littorea* based on the morphology of flower. Based on the analysis of two genes NGFI-A binding (*nab*) and pentatricopeptide repeat (*ppr*), Guo et al., [13] also indicated that *Lumnitzera* x *rosea* was hybrid of *Lumnitzera racemosa* (mother) and *L. littorea* (father). In Vietnam, *Lumnitzera* x *rosea* was recorded at Hue city and Ha Tien, Kien Giang province. Today, phylogenetic analysis of mangroves was also carried out by several authors. However, the authors have just focused on the specific families, such as Rhizophoraceae [14], Euphorbiaceae [15], Acanthaceae [16], Avicenniaceae [17]. There is no phylogenetic analysis for the full mangrove trees. Moreover, which genetic markers should be used as DNA barcodes for mangroves is a challenge for science. Hence, the aims of this study are to answer the following questions: i) Can the ITS sequences in the nuclear be the DNA barcodes for mangrove? and ii) Where is the position of *Lumnitzera* x *rosea* in the phylogenetic tree of mangroves?

MATERIALS AND METHODS

Sample collection

Specimens including *Lumnitzera littorea*, *L. racemosa* and *L.* x *rosea* were collected at Hue (*Lumnitzera littorea*, *L.* x *rosea*), Ninh Hoa (*L. racemosa*), Cam Ranh, Khanh Hoa province (*Lumnitzera littorea*). Sampling sites were shown in fig. 1. For each sample, the small young leaves were cut from plants, then dried by silica gel. In the same plants, the samples of plant were also collected for herbarium voucher specimens. The herbarium voucher specimens are now stored at Department of Marine Botany, Institute of Oceanography, Vietnam.

DNA extraction and polymerase chain reaction (PCR)

Dry samples were homogenized using a bead mill (22 Hz, 2 min, MM400, Retsch GmbH, Haan, Germany), and 100 mg of the finely powdered plant material was used for DNA extraction. DNA extraction was carried out using the Plant Nucleospin II Kit (Macherey & Nagel, Duren, Germany) following manufacturer's instructions with slight modifications. DNA quality was checked on agarose gels stained with ethidium bromide or Midori green, and the concentration was measured by a microplate reader with micro-volume plates (Synergy MxMulti-Mode, BioTek, Germany). The region selected for PCR amplification was the nuclear ITS region including the 5.8S region. Primer pairs used were P674 (5'-CCTTATCATTTAGAGGAAGGAG-3') and P675 (5'-TCCTCCGCTTATTGATATGC-3') to amplify a sequence of 700 bp to 720 bp consisting of ITS1, 5.8S and ITS2. The total volume of 25mL included 1x DreamTaq Green buffer, 0.2 mM dNTPs, 2 mM MgCl2, 1U *Taq* polymerase (MBI Fermentas, St. Leon-Rot, Germany), 10 ng to 30 ng template DNA and 1 pmol of each primer. PCR was performed in a PTC 200 thermocycler (Biozym-Diagnostik GmbH, Hess. Oldendorf, Germany) with a heated lid under the following conditions: initial denaturation (4 min at 95^{o}C), followed by 30 cycles of denaturation (25 s at 95^{o}C), primer annealing (30 s at 52^{o}C), extension (35 s at 72^{o}C) and termination by a final hold at 10^{o}C. All PCR reactions were repeated two to four times independently with the same specimen to keep errors (possibly created by the *Taq* polymerase) in the final consensus

sequence to a minimum. Direct sequencing of the PCR product was done by GATC Biotech (Konstanz, Germany) using primer P674 or P675 for both directions.

Bioinformatics analysis

The consensus sequence was achieved by Clone Manager 9 (Sci-Ed, Cary, NC, USA). Four ITS sequences from three species (from this study) and 55 ITS sequences of mangroves were retrieved from the GenBank (table 1). These sequences were aligned by CLUSTAL W using MEGA6 [18], and the alignment was further modified by eye. Gaps were considered as missing data. Identical sequences within each species were excluded from the alignment. jModelTest [19] and the corrected AIC were used to find the best model for the analysis. Phylogenetic analyses were performed using Maximum Likelihood (ML) in RAxML version 8.1 [20] with the model Tamura-Nei+G [21], Neighbour Joining (NJ) with the model TrN+G, Maximum Parsimony (MP) [22] in MEGA6 and Bayesian Inference (BI) (Metropolis Coupled Markov-chain Monte-Carlo method, GTR+G model) performed in MrBayes v.3.2.2 [23]. In the BI, the two parallel runs with four chains each (three heated and one cold) were performed for 1 million generations, sampling a tree every 100 generations. Only trees sampled after convergence were used to make inferences about the phylogeny and to compute a 50% majority-rule consensus tree. The posterior probability values in each node were calculated by FigTree software (version 1.4.2) [24]. In the analyses, trees were tested by the bootstrapping method with 1,000 replications. Sequence divergences were also calculated in MEGA6. The consensus tree based on four different trees (achieved from the four methods) was constructed by Dendro Scope software, version 3.2.10 [25]. Comparison between two phylogenetic trees was also performed by the tanglegram option in the Dendro Scope software.

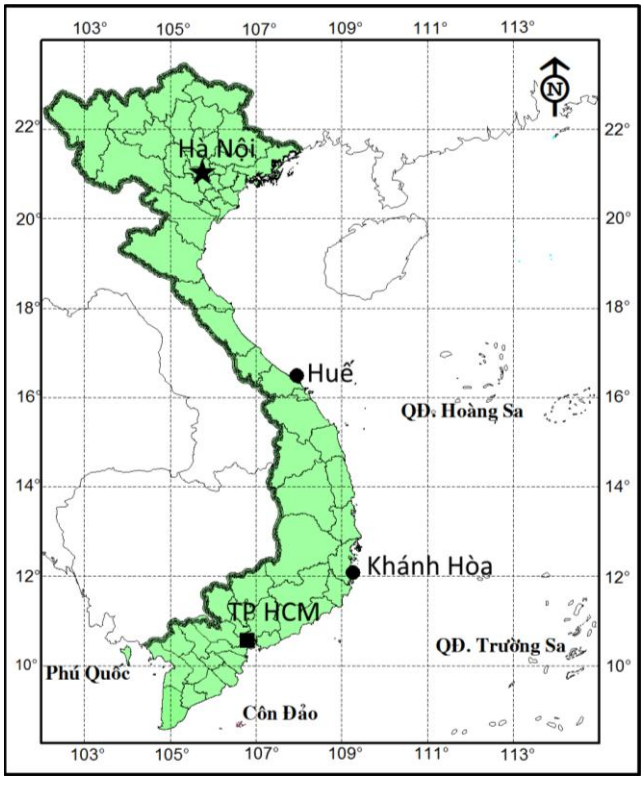

Fig. 1. The map of Vietnam and study sites (the black solid rounds). The map was processed by MapInfor Pro™, version 12.5.5 (Pitney Bowes Software Inc., NY, USA)

Table 1. List of taxa and GenBank (GB) number used for the analysis: (-) direct submission; *: Sample collected at Hue; **: Samples collected at Khanh Hoa

No	Families	Species	Markers and GB number		
			*rbc*L	*mat*K	ITS
1	Acanthaceae	*Acanthus ilicifolius*			FJ976671
2		*Avicennia bicolor*			EF540989
3		*Avicennia marina*	JX572318	AB114527	AF477770
4		*Avicennia marina*			AF477771
5		*Avicennia marina* subsp. *australasica*			AF365978
6		*Avicennia germinans*	JQ590029	AF531771	EF136921
7	Avicenniaceae	*Avicennia germinans*			EF136922
8		*Avicennia germinans*			DQ469854
9		*Avicennia germinans*			DQ469853
10		*Avicennia germinans*			EF136923
11		*Avicennia schaueriana*			EF540986
12		*Avicennia schaueriana*			DQ469862
13	Bombaceae	*Camptostemon schultzii*	AF022120	AY321162	HQ658360
14		*Conocarpus erectus*			AY050562
15		*Laguncularia racemosa*			AF425686
16		*Laguncularia racemosa*			AF425685
17		*Lumnitzera littorea**			
18	Combretaceae	*Lumnitzera littorea***			
19		*Lumnitzera littorea*			AF160468
20		*Lumnitzera racemosa***			
21		*Lumnitzera racemosa*	FJ381827	AB114599	AF160467
22		*Lumnitzera* x *rosea**			
23	Euphorbiaceae	*Excoecaria agallocha*			KC460266
24	Loranthaceae	*Amyema queenslandica*			DQ333834
25	Lythraceae	*Pemphis acidula*			AY035762
26	Meliaceae	*Xylocarpus moluccensis*			FJ518907
27	Myrsinaceae	*Aegiceras corniculatum*			FJ976669
28	Myrtaceae	*Osbornia octodonta*			EF041844
29	Pellicieraceae	*Pelliciera rhizophorae*	AF421099	AJ429303	AY452670
30		*Pelliciera* sp			AY348856
31	Rhizophoraceae	*Bruguiera cylindrica*	AF127694	AF329464	HM366078
32		*Bruguiera cylindrica*			HM366079
33		*Bruguiera gymnorhiza*	JX905966	AB233823	HM366082
34		*Bruguiera gymnorhiza*			HM366083
35		*Bruguiera hainesii*			EU000399
36		*Bruguiera parviflora*			HM366110
37		*Bruguiera parviflora*			HM366111
38		*Bruguiera sexangula*	AF127682	AF329464	HM366122
39		*Bruguiera sexangula*			HM366134
40		*Ceriops tagal*	AF127684	AF105089	AF130329
41		*Ceriops tagal*			EF119031
42		*Kandelia candel*	AF127682	AB114567	AF130327
43		*Kandelia candel*			EF119071
44		*Rhizophora apiculata*	KC119043	KC119042	HQ337924
45		*Rhizophora apiculata*			HQ337918
46		*Rhizophora apiculata*			HQ337923
47		*Rhizophora mangle*	JX664070	JX661962	HQ337955
48		*Rhizophora mangle*			HQ337958
49		*Rhizophora mucronata*	KF147509	AF329465	HQ337948
50		*Rhizophora mucronata*			HQ337947
51		*Rhizophora mucronata*			HQ337946
52		*Rhizophora stylosa*	AB668371	AF105092	HQ337938
53		*Rhizophora stylosa*			HQ337935
54		*Rhizophora stylosa*			HQ337934
55	Sonneratiaceae	*Sonneratia alba*			AY680878
56		*Sonneratia caseolaris*			AY674897
57		*Sonneratia griffithii*			KJ511912
58		*Sonneratia ovata*			AY674934
59		*Sonneratia ovata*			AF163702

RESULTS AND DISCUSSION

Phylogenetic analysis based on its sequences

A final alignment of 669 bp (including gaps) was generated for ITS1-5.8S-ITS2, in which 420 were parsimony informative characters, 37 were singleton sites, 180 were conserved sites, and 458 were variable sites. Results of the four algorithms applied (ML, NJ, MP, BI) showed that all sequences were distributed into 13 clades consisting of Meliaceae (Clade I), Bombaceae (Clade II), Rhizophoraceae (Clade III), Loranthaceae (Clade IV), Myrtaceae (Clade V), Combretaceae (Clade VI), Lythraceae (Clade VII), Sonneratiaceae (Clade VIII), Myrsinaceae (Clade IX), Euphorbiaceae (Clade X), Pellicieraceae (Clade XI), Acanthaceae (Clade XII), and Avicenniaceae (Clade XIII) (fig. 2). There was no difference in the topology of the phylogenetic trees based on these different methods except for small differences in the bootstrap values.

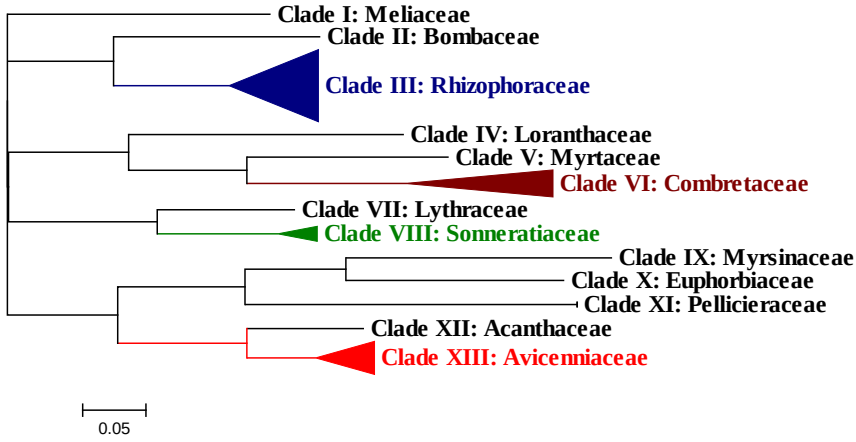

Fig. 2. Phylogeny of members of mangroves inferred from Maximum Likelihood, Neighbour Joining, Maximum Parsimony and Bayesian Inference. The data set is based on 699 bp (including gaps) of nrDNA sequences comprising ITS-1, 5.8SrDNA and ITS-2. The bootstrap and posterior probability values of each method are shown in each node: above nodes, left: Maximum Likelihood, right: Maximum Parsimony; below nodes, left: Neighbour Joining, right: Bayesian Inference

For mangroves, four families including Rhizophoraceae, clade III; Combretaceae, clade VI; Sonneratiaceae, clade VIII; and Avicenniaceae, clade XIII showed higher species numbers. The detailed analysis of Rhizophoraceae depicted four sub-clades. They are genera *Rhizophora*, *Bruguiera*, *Ceriops*, and *Kandelia* with very high bootstrap (>75%) and posterior probability values (1.0). All species in this family are resolved. For the genus *Rhizophora*, *Rhizophora mucronata* Lam., *R. apiculata* Blume., *R. mangle* Lam. and *R. stylosa* were distributed in four distinct groups with higher than 75% bootstrap values and 0.75 posterior probability values. Our result was supported by the previous study based on single *rbc*L [26, 27], or single *mat*K [28], or combination of ITS and *mat*K [29] when Rhizophoraceae was formed from four clades, or four genera. For the species resolution, the single *rbc*L did not resolve members of *Rhizophora*. Shi et al., [29] also depicted that species resolution of *mat*K was not clear for three species including *Rhizophora mucronata*, *R. stylosa* and *R. apiculata*. In the same way, *Bruguiera parviflora* (Roxfb.) W. & A. *ex* Griff, *B. cylindrical* (Lam.) Bl., *B. sexangula* (Lour.) Poir., *B. gymnorhiza* and *B. hainesii* formed five distinct groups in the genus *Bruguiera* with bootstrap values and posterior probability values higher than 50% and 0.98 (fig. 3).

Hence, using ITS sequence as DNA barcodes for Rhizophoraceae is more suitable than *rbc*L and *mat*K in comparison to the previous studies.

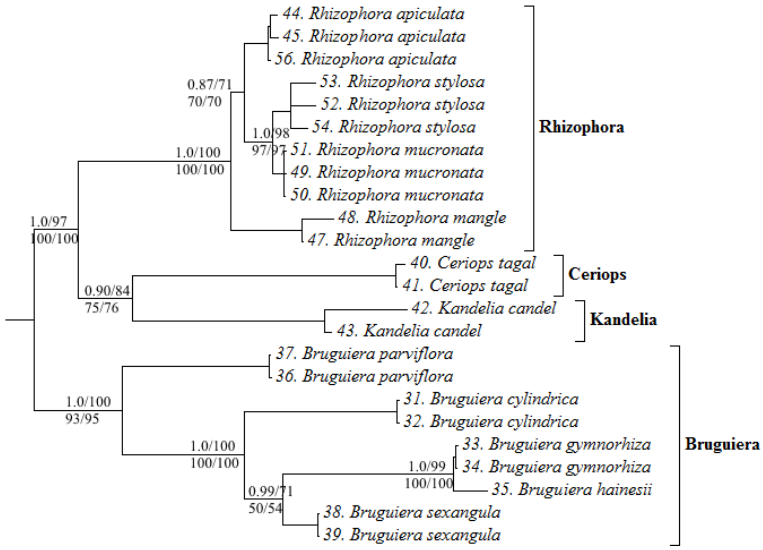

Fig. 3. Phylogeny of members of clade III - Rhizophoraceae (fig. 2). See fig. 2 for the information of methods. The font numbers of each taxon are the numbers in table 1

In the Combretaceae family, three sub-clades were formed, they are *Conocarpus*, *Lumnitzera* and *Laguncularia*. Genus *Lumnitzera* was just divided into two groups instead of three groups with three species: *Lumnitzera littorea*, *L. racemosa* and the hybrid *L.* x *rosea*. Our analysis showed that *L.* x *rosea* did not form a distinct group, it clustered to *Lumnitzera littorea*. The analysis of genus *Sonneratia* (Sonneratiaceae) also found that four species including *Sonneratia ovata* Backer, *S. griffithii* Kurz, *S. alba* Sm. and *S. caseolaris* (L.) Engl. formed four distinct groups with bootstrap values and posterior probability higher than 90% and 1.0 respectively (fig. 4).

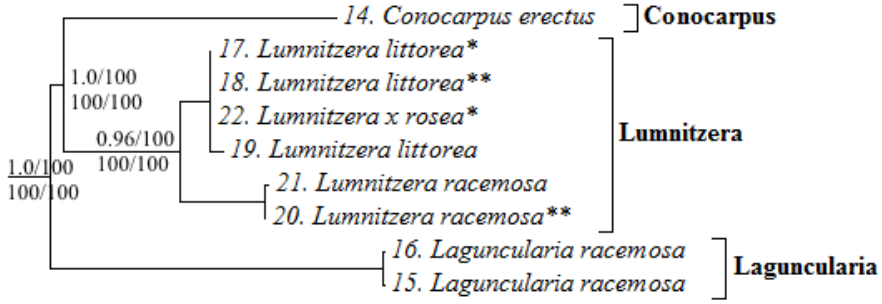

Fig. 4. Phylogeny of members of clade VI - Combretaceae. See fig. 2, 3 for the information

Hybridization between two species is the common phenomenon in the nature, for mangrove, species *Sonneratia* x *gulngai* N. C. Duke is the hybrid between *S. alba* and *S. caseolaris* or hybridization between *Sonneratia alba* and *S. griffithii* to form a new species [30]. In *Rhizophora* genus, *Rhizophora* x *harrisonii* is hybrid species of *R. mangle* and *R. racemosa* G. Mey. [31] or there is hybridization between *R. mucronata* và *R. stylosa* [32]. Hybridization between two members of *Lumnitzera* was first reported by Tomlinson et al., [12] that was

based on the description of flower morphology. This study found that *Lumnitzera* x *rosea* clustered into *Lumnitzera littorea*. Our result was supported by Guo et al., [13] when the author found that one sample of *Lumnitzera* x *rosea* clustered into *L. littorea*, and another sample clustered into *L. racemosa* based on two genes: *nab* and *ppr*. For other marine macrophytes, for example in seagrasses species, hybridization also occurred between two species *Halodule uninervis* (Forssk.) Boiss. and *H. pinifolia* (Miki) Hartog [33]. Based on analysis of *phy*B sequences, the authors indicated that some samples clustered to *Halodule uninervis* group while other samples clustered to *Halodule pinifolia* group. Unfortunately, our surveys just collected a unique sample at Hue city, there are no more samples for comparison. Hence, our next studies will try to collect *Lumnitzera* x *rosea* at Ha Tien, Kien Giang province as reported by Duke [34] for analysis.

For the genus *Avicennia* (Avicenniaceae), the result of the analysis showed that *Avicennia schaueriana* Stapf & Leechman ex Moldenke, *A. bicolor* Standl., *A. germinans* (L.) L., and *A. marina* (Forsk.) Vierh. clustered into four distinct groups. Interestingly, *Avicennia marina* subsp. *australisica* (Walp.) J. Everett formed a distinct group from *A. marina* with bootstrap values and the posterior probability of 100% and 1.0 respectively. Tomlinson [12] depicted that the polymorphism within species of *Avicennia* was very high. Morphological variants within one species may lead to the misidentification of taxa, as the report of Saenger and Brooks [35], Said and Ehsan [36] in the case of *Avicennia marina* or *A. germinans* [37]. Our analysis on ITS was also supported by Nettel et al., [38], Schwarzbach and McDade [28] and Shi et al., [29] when ITS showed higher species resolution in *Avicennia*. Hence, using ITS sequence as DNA barcode for this genus also has more advantages.

The evolutionary divergence among species of genus *Rhizophora* was presented in table 1. *p*-distance between *Rhizophora mucronata* and *R. stylosa* is 0.011 - 0.013 which is less than other members of genus (0.044 - 0.016). For the *Bruguiera* genus, the *p*-distance between *B. hainesii* and *B. gymnorhiza* is 0.007, much lower than *p*-distance between *B. hainesii* and *B. cylindrical* (0,062). The *p*-distances between *L.* x *rosea* and *Lumnitzera littorea* is 0. In contrast, the *p*-distance between *Lumnitzera littorea* and *L. racemosa* is 0.05 - 0.055. The *p*-distance among members of *Sonneratia* are 0.047 - 0.059. The *p*-distance between *A. marina* subsp. *australisica* and *A. marina* is 0.04 which is much lower than other members of *Avicennia* (table 2).

Table 2. Evolutionary divergence among species of Combretaceae: *Ll = Lumnitzera littorea, Lxr = Lumnitzera* x *rosea, Lr= Lumnitzera racemosa, Lar = Laguncularia racemosa,* H = Hue, KH = Khanh Hoa. See table 2 for the method of calculation

	Ll	*Ll* H	*Lxr*	*Ll* KH	*Lr* KH	*Lr*	*Lar*	*Lar*	*Ce*
Ll									
Ll H	0.006								
Lxr	0.006	0.000							
Ll KH	0.006	0.000	0.000						
Lr KH	0.055	0.050	0.050	0.050					
Lr	0.053	0.051	0.051	0.051	0.002				
Lar	0.186	0.180	0.180	0.180	0.189	0.191			
Lar	0.186	0.180	0.180	0.180	0.189	0.191	0.000		
Ce	0.176	0.171	0.171	0.171	0.186	0.188	0.204	0.204	

Comparison of species resolution to the previous dna barcodes

The results of the ITS analysis and the results obtained from the *rbc*L/*mat*K, barcode system were compared by a tanglegram tree (fig. 5). The result indicated that species resolution of ITS marker was higher than

concatenated *rbc*L/*mat*K sequences. In detail, in the case of Rhizophoraceae, the concatenated *rbc*L/*mat*K showed four clades that are four genera. However, the bootstrap values and posterior probability were too low. Species resolution among three species *Rhizophora stylosa*, *R. mucronata* and *R. apiculata* was very low (fig. 5, panel A) whereas ITS analysis (fig. 5, panel B) strongly supported it. For other families/genera, there is no difference between two barcode systems.

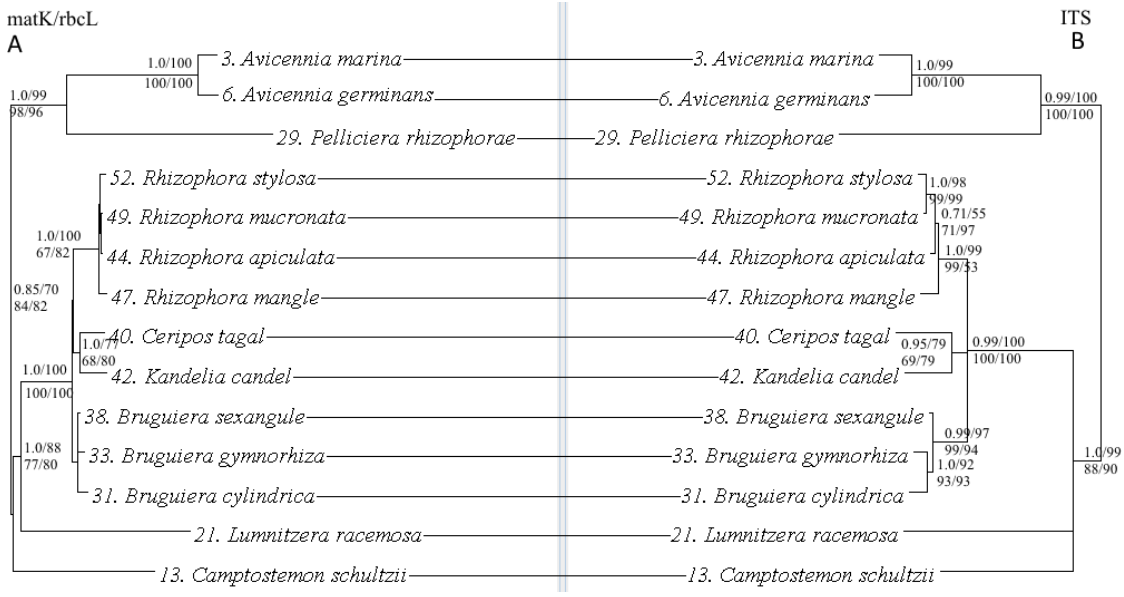

Fig. 5. Tanglegram of consensus phylogenetic trees based on two barcode systems and single ITS sequence. Panel A is phylogenetic trees based on 1,477 bp (including gaps) on two sequences *rbc*L and *mat*K. Panel B is phylogenetic trees based on 699 bp (including gaps) of ITS sequences. See fig. 2 for the methods and legend. The tanglegram phylogenetic tree was constructed by method Tanglegram in DendroScope software [25]

The ITS marker is clearly more advantageous than *rbc*L/*mat*K, and it overcomes the disadvantages of *rbc*L/*mat*K barcodes. According to Lucas et al., [39], the disadvantages of the *rbc*L/*mat*K approach were: i) Using two primer pairs for PCR of *mat*K; (ii) Costly and time-consuming due to using cloning vector and carrying out the cloning steps; (iii) Not full species resolution; and (iv) Forming long concatenated sequences (1,477 bp) that increase and prolong steps in the bioinformatics analyses. In contrast, using ITS as DNA barcode showed a number of advantages: (i) One PCR condition can be applied to all samples; (ii) Cheap and fast; (iii) It can resolve the closely related species.

In this study, we focused on the main families of mangroves including Rhizophoraceae, Combretaceae, Sonneratiaceae, and Avicenniaceae. This research may be the basis for the next studies with the bigger scale such as all mangrove species in Vietnam.

CONCLUSION

The results and discussion presented above prove that i) Species resolution based on ITS sequences is higher than concatenated *rbc*L and *mat*K, ii) *Lumnitzera* x *rosea* and *Lumnitzera littorea* cluster into the same group, and finally iii) ITS should be used as DNA barcodes for mangrove instead of the concatenated *rbc*L and *mat*K system.

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REFERENCES

1. Duke, N. C., 2013. Mangrove floristics and biogeography. In: *Robertson, A. I., and Alongi, D. M., (eds). Tropical mangrove ecosystems. American Geophysical Union*, Pp. 63-100.
2. Ng, W. L., Chan, H. T., and Szmidt, A. E., 2013. Molecular identification of natural mangrove hybrids of *Rhizophora* in Peninsular Malaysia. *Tree genetics & Genomes*, **9**(5), 1151-1160.
3. Waugh, J., 2007. DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, **29**(2), 188-197.
4. Group, C. P. W., Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., ... and Fazekas, A. J., 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, **106**(31), 12794-12797.
5. Chen, C. W., Huang, Y. M., Kuo, L. Y., Nguyen, Q. D., Luu, H. T., Callado, J. R., Farrar, D. R., and Chiou, W. L., 2013. *trnL-F* is a powerful marker for DNA identification of field vittarioid gametophytes (Pteridaceae). *Annals of Botany*, **111**(4), 663-673.
6. Jing, Y. U., Jian-Hua, X. U. E., and Shi-Liang, Z. H. O. U., 2011. New universal matK primers for DNA barcoding angiosperms. *Journal of Systematics and Evolution*, **49**(3), 176-181.
7. De Groot, G. A., During, H. J., Maas, J. W., Schneider, H., Vogel, J. C., and Erkens, R. H., 2011. Use of *rbc*L and *trn*L-F as a two-locus DNA barcode for identification of NW-European ferns: an ecological perspective. *PLoS One*, **6**(1), e16371.
8. Vy, N. X., Holzmeyer, L., and Papenbrock, J., 2013. New record of the seagrass species *Halophila major* (Zoll.) Miquel in Vietnam: evidence from leaf morphology and ITS analysis. *Botanica Marina*, **56**(4), 313-321.
9. Nguyen, V. X., Detcharoen, M., Tuntiprapas, P., Soe-Htun, U., Sidik, J. B., Harah, M. Z., Prathep, A., and Papenbrock, J., 2014. Genetic species identification and population structure of *Halophila* (Hydrocharitaceae) from the Western Pacific to the Eastern Indian Ocean. *BMC Evolutionary Biology*, **14**(1), 92.
10. Nguyen, X. V., Höfler, S., Glasenapp, Y., Thangaradjou, T., Lucas, C., and Papenbrock, J., 2015. New insights into DNA barcoding of seagrasses. *Systematics and Biodiversity*, **13**(5), 496-508.
11. Hong, P. N., and San, H. T., 1993. *Mangroves of Vietnam* (Vol. 7). *Dyan Print Ltd., Bangkok, Thailand*.
12. Tomlinson, P. B., Bunt, J. S., Primack, R. B., and Duke, N. C., 1978. *Lumnitzera rosea* (Combretaceae) - its status and floral morphology. *Journal of the Arnold Arboretum*, **59**(4), 342-351.
13. Guo, M., Zhou, R., Huang, Y., Ouyang, J., and Shi, S., 2011. Molecular confirmation of natural hybridization between *Lumnitzera racemosa* and *Lumnitzera littorea*. *Aquatic Botany*, **95**(1), 59-64.
14. Schwarzbach, A. E., and Ricklefs, R. E., 2000. Systematic affinities of Rhizophoraceae and Anisophylleaceae, and intergeneric relationships within Rhizophoraceae, based on chloroplast DNA, nuclear ribosomal DNA, and morphology. *American Journal of Botany*, **87**(4), 547-564.
15. Das, A. B., Jena, S., Pradhan, C., and Chand, P. K., 2011. Genetic variability among male populations of a minor mangrove *Excoecaria agallocha* L. as evident by chromosome morphology and DNA markers. *The Nucleus*, **54**(1), 39-47.
16. Lakshmi, M., Rajalakshmi, S., Parani, M., Anuratha, C. S., and Parida, A., 1997. Molecular phylogeny of mangroves I. Use of molecular markers in assessing the intraspecific genetic variability in the mangrove species *Acanthus ilicifolius* Linn. (Acanthaceae). *Theoretical and Applied Genetics*, **94**(8), 1121-1127.

17. Salas-Leiva, D. E., Mayor-Durán, V. M., and Toro-Perea, N., 2009. Genetic diversity of black mangrove (*Avicennia germinans*) in natural and reforested areas of Salamanca Island Parkway, Colombian Caribbean. *Hydrobiologia*, **620**(1), 17-24.

18. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, **30**(12), 2725-2729.

19. Darriba, D., Taboada, G. L., Doallo, R., and Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**(8), 772.

20. Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**(9), 1312-1313.

21. Tamura, K., and Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, **10**(3), 512-526.

22. Felsenstein, J., 1992. Phylogenies from restriction sites: a maximum-likelihood approach. *Evolution*, **46**(1), 159-173.

23. Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3), 539-542.

24. Rambaut, A., 2015. FigTree (version 1.4.2). University of Edinburgh, Scotland. Retrieved from http://tree.bio.ed.ac.uk/software/figtree/ (accessed 27 August, 2016).

25. Huson, D. H., and Scornavacca, C., 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Systematic Biology*, **61**(6), 1061-1067.

26. Maurin, O., Davies, T. J., Yessoufou, K., Daru, B. H., Bezeng, B. S., Mankga, L. T., and Van der Bank, M., 2013. Human population density correlates with phylogenetic diversity and plant vulnerability in southern Africa. *South African Journal of Botany*, *86*, 166.doi:10.1016/j.sajb.2013.02.104

27. Takayama, K., Tamura, M., Tateishi, Y., Webb, E. L., and Kajita, T., 2013. Strong genetic structure over the American continents and transoceanic dispersal in the mangrove genus Rhizophora (Rhizophoraceae) revealed by broad-scale nuclear and chloroplast DNA analysis. *American Journal of Botany*, **100**(6), 1191-1201.

28. Schwarzbach, A. E., and McDade, L. A., 2002. Phylogenetic Relationships of the Mangrove Family Avicenniaceae Based on Chloroplast and Nuclear Ribosomal DNA Sequences. *Systematic Botany*, **27**(1), 84-98.

29. Shi, S., Du, Y., Boufford, D. E., Gong, X., Huang, Y., He, H., and Zhong, Y., 2003. Phylogenetic position of *Schnabelia*, a genus endemic to China: Evidence from sequences of cpDNA *mat*K gene and nrDNA ITS regions. *Chinese Science Bulletin*, **48**(15), 1576-1580.

30. Suo, Q. I. U., Ren-Chao, Z. H. O. U., Yun-Qin, L. I., Sonjai HAVANOND, C. J., and Su-Hua, S. H. I., 2008. Molecular evidence for natural hybridization between Sonneratia alba and *S. griffithii*. *Journal of Systematics and Evolution*, **46**(3), 391-395.

31. Cerón-Souza, I., Rivera-Ocasio, E., Medina, E., Jiménez, J. A., McMillan, W. O., and Bermingham, E., 2010. Hybridization and introgression in New World red mangroves, *Rhizophora* (Rhizophoraceae). *American Journal of Botany*, **97**(6), 945-957.

32. Ng, W. L., and Szmidt, A. E., 2015. Introgressive hybridization in two Indo-West Pacific *Rhizophora* mangrove species, *R. mucronata* and *R. stylosa*. *Aquatic Botany*, **120**, 222-228.

33. Ito, Y., and Tanaka, N., 2011. Hybridisation in a tropical seagrass genus, *Halodule* (Cymodoceaceae), inferred from

plastid and nuclear DNA phylogenies. *Telopea*, **13**(1-2), 219-231.

34. Duke, N., 2012. Mangroves of the Kien Giang Biosphere Reserve Viet Nam. *Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Rach Gia city*.
35. Saenger, P., and Brooks, L., 2008. Phenotypic leaf variation in *Avicennia marina* in tropical Australia: can discrete subpopulations be recognised in the field?. *Australian Journal of Botany*, **56**(6), 487-492.
36. Said, W. M., and Ehsan, N. O., 2010. Morphological and molecular evidences among four heteroforms of *Avicennia marina* (Forssk) Vierh. *Journal of American Science*, **6**(11), 843-856.
37. Osland, M. J., Day, R. H., Larriviere, J. C., and From, A. S., 2014. Aboveground allometric models for freeze-affected black mangroves (*Avicennia germinans*): equations for a climate sensitive mangrove-marsh ecotone. *PloS One*, **9**(6), e99604.
38. Nettel, A., Dodd, R. S., Afzal-Rafii, Z. A. R. A., and Tovilla-Hernández, C. R. I. S. T. I. A. N., 2008. Genetic diversity enhanced by ancient introgression and secondary contact in East Pacific black mangroves. *Molecular Ecology*, **17**(11), 2680-2690.
39. Lucas, C., Thangaradjou, T., and Papenbrock, J., 2012. Development of a DNA barcoding system for seagrasses: successful but not simple. *PLoS One*, **7**(1), e29987.

LỰA CHỌN CHỈ THỊ PHÂN TỬ CHO ĐỊNH LOẠI CÂY NGẬP MẶN BẰNG PHƯƠNG PHÁP MÃ VẠCH AND

Nguyễn Xuân Vỵ[1], Nguyễn Nhật Như Thủy[1], Nguyễn Trung Hiếu[1], Lương Quang Đốc[2]

[1]Phòng Thực vật biển, Viện Hải dương học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
[2]Khoa Sinh học, Trường Đại học Khoa học, Đại học Huế

TÓM TẮT: Trong phân loại thực vật, phương pháp so sánh hình thái được xem là chính yếu nhưng thường gặp khó khăn vì đòi hỏi tính nguyên vẹn, đầy đủ các thành phần của mẫu vật. Bên cạnh đó, phương pháp này nhiều khi gặp khó khăn khi các đặc điểm hình thái không có sự khác biệt rõ hoặc mang tính trung gian. "DNA barcoding" là một phương pháp dựa trên trình tự ngắn của gen trực giao hay còn gọi là 'DNA barcodes' và chỉ cần một lượng mẫu rất nhỏ cho phân tích. Nghiên cứu này dựa trên 59 trình tự ITS của cây ngập mặn bao gồm 55 trình từ lấy Ngân hàng gen và 4 trình tự có được từ ba loài thuộc chi Cóc (*Lumnitzera*) thu tại Huế và ven bờ Khánh Hòa. Bốn phương pháp xây dựng cây phát sinh chủng loại bao gồm Maximum Likelihood, Neighbor Joining, Maximum Parsimony và Bayesian Inference được áp dụng. Kết quả nghiên cứu chỉ ra rằng mức độ định loại đến loài của chỉ thị ITS cao hơn sự kết hợp hai gen *rbc*L và *mat*K như đề xuất trước đó. Loài cóc hồng thu tại Huế không có khác biệt với loài cóc đỏ dựa trên chỉ thị ITS. Chỉ thị ITS nên được sử dụng như là DNA barcodes cho cây ngập mặn thay vì kết hợp hai gen *rbc*L và *mat*K.

Từ khóa: DNA barcodes, cây ngập mặn, ITS, *Lumnitzera*.

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