



Evaluation of Multilocus Marker Efficacy for Delineating Mangrove Species of West Coast India

Ankush Ashok Saddhe, Kundan Kumar*

Department of Biological Sciences, Birla Institute of Technology & Science Pilani, K. K. Birla Goa Campus, Goa-403726, India

Abstract

Background: The Plant DNA barcoding is complex and require more than one marker(s) compared to animal barcoding. Mangroves are diverse estuarine ecosystem prevalent at tropical and subtropical zone, but anthropogenic activity turned them into vulnerable ecosystem. For conservation strategies, there is a need to build molecular reference library based on molecular marker alongwith morphological characteristics

Result: In this study, we tested the core plant barcode (*rbcL* + *matK*) and four promising complementary barcodes (ITS2, *psbK-psbI*, *rpoC1* and *atpF-atpH*) in representative 14 mangroves species belonging to 5 families from west coast India. Data analysis was performed based on barcode gap analysis, intra- and inter-specific genetic distance, Automated Barcode Gap Discovery (ABGD), TaxonDNA (BM, BCM), Poisson Tree Processes (PTP) and General Mixed Yule-coalescent (GMYC). The *rbcL* locus showed highest PCR efficiency and sequencing success (100%) rate, followed by ITS2 and *matK*. Using a single locus for analysis, ITS2 exhibited the highest discriminatory power (87.82%) but combinations of *matK* + ITS2 provided the highest discrimination success (89.74%) rate except *Avicennia* genus. Single ITS2 barcode locus resolved *Rhizophora apiculata*, and *R. mucronata* based on GMYC analysis and *Sonneratia* species were demarcated using ABGD tools with relative gap width (X=1.5). Further we evaluated 3 additional markers (*psbK-psbI*, *rpoC1* and *atpF-atpH*) for *Avicennia* genera (*A. alba*, *A. officinalis* and *A. marina*) and out of which *atpF-atpH* locus was able to discriminate three species of *Avicennia* genera based on ABGD and TaxonDNA analysis

Significance: Our analysis underscored the efficacy of *matK*+ITS2 markers with *atpF-atpH* as the best combination for mangrove identification of west coast India region.

Key Words: DNA Barcoding, West Coast of India, *rbcL*, *matK*, ITS2

Results

Mangroves Species

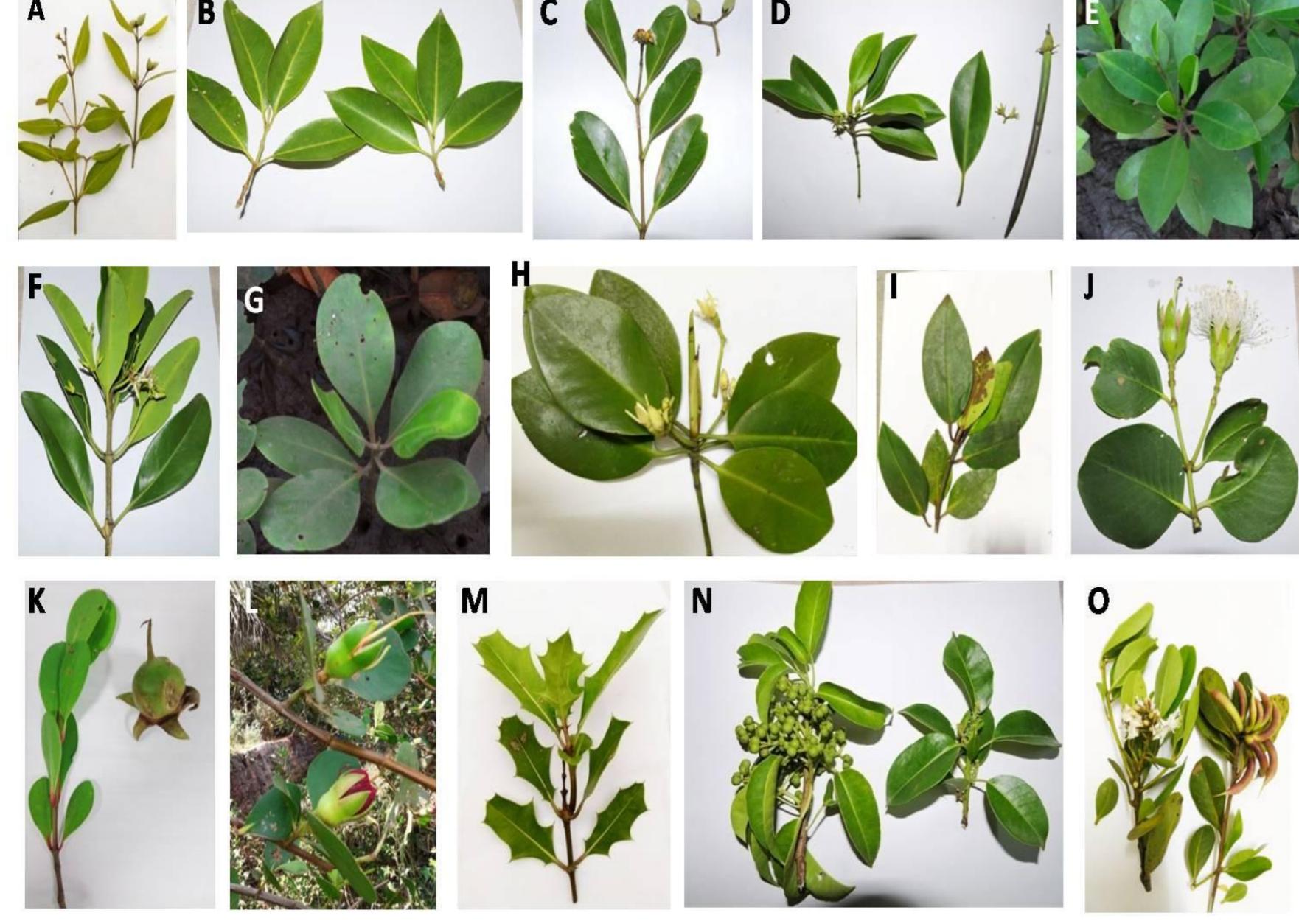


Fig. 1 Photos of 14 mangroves species used in our study (A) *Avicennia marina* (B) *A. alba* (C) *A. officinalis* (D) *Bruguiera cylindrica* (E) *B. gymnorhiza* (F) *Kandelia candel* (G) *Ceriops tagal* (H) *Rhizophora mucronata* (I) *R. apiculata* (J) *Sonneratia alba* (K, L) *S. caseolaris* (M) *Acanthus ilicifolius* (N) *Excoecaria agallocha* and (O) *Aegiceras corniculatum*.

Genetic Divergence Table

Barcode	Level	N	Taxa	Comparison	Min Dist (%)	Mean Dist (%)	Max Dist (%)	SE Dist (%)
<i>rbcL</i>	Species	44	14	50	0	0.24	0.68	0
	Genus	26	4	53	0	0.35	0.68	0
	Family	29	2	132	1.71	2.63	4.01	0
<i>matK</i>	Species	45	14	50	0	0.2	1.32	0.01
	Genus	25	4	45	0	0.9	2.32	0.02
	Family	29	2	141	2.11	5.82	13.37	0.02
ITS2	Species	40	14	39	0	1.85	16.75	0.1
	Genus	25	4	45	0	5.8	35.14	0.25
	Family	28	2	133	5.72	12.35	40.26	0.08
<i>matK</i> + ITS2	Species	39	14	37	0	0.51	4.02	0.02
	Genus	24	4	43	0	1.76	7.84	0.05
	Family	28	2	133	3.35	7.39	19.89	0.03
<i>atpF-atpH</i>	Species	9	3	9	0	0.11	0.6	0.02
	Genus	9	1	27	0.39	1.03	1.62	0.02
<i>psbK-psbI</i>	Species	6	2	6	0	1.63	3.85	0.27
	Genus	6	1	9	0.96	2.16	4.94	0.14
<i>rpoC1</i>	Species	6	2	6	0.22	0.37	0.67	0.03
	Genus	6	1	9	0	0.3	0.67	0.02

Table 1. Distribution of sequence divergence at the species, genus and family level (Distance summary result - BOLD system). N - Number of sequences. Min Dist – Minimum Distance, Max Dist – Maximum Distance

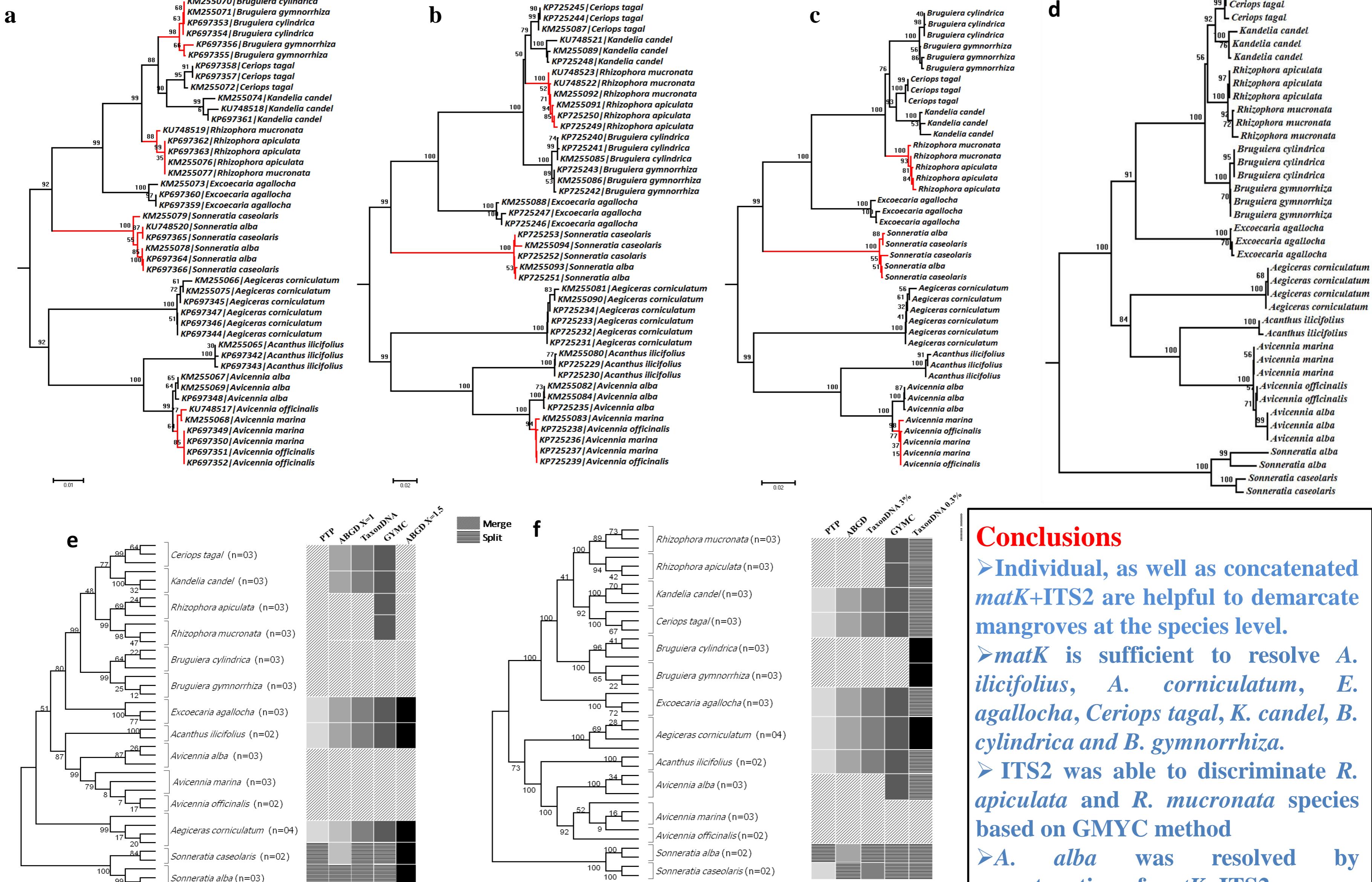


Fig. 2 Neighbor joining tree (Kimura 2 Parameter) and Bayesian phylogenetic tree. a) *rbcL*, b) *matK*, c) *matK*+*ITS2* concatenated NJ (K2P) trees. Highlighted clades (red color) indicate unresolved or least differentiate mangroves sequences (e) ITS2 and (f) *matK*+*ITS2* gene. Vertical boxes on the right indicate the clades detected by the coalescent-based GMYC, PTP, the distance-based ABGD and TaxonDNA methods.

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(Email ID- kundan@goa.bits-pilani.ac.in)

Conclusions

Individual, as well as concatenated *matK*+*ITS2* are helpful to demarcate mangroves at the species level.

matK is sufficient to resolve *A. ilicifolius*, *A. corniculatum*, *E. agallocha*, *Ceriops tagal*, *K. candel*, *B. cylindrica* and *B. gymnorhiza*.

ITS2 was able to discriminate *R. apiculata* and *R. mucronata* species based on GMYC method

A. alba was resolved by concatenation of *matK*+*ITS2*.

A cryptic genus *Avicennia* was delimitated based on the *atpF-atpH* single barcode.