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Unveiling the molecular mechanisms of intertidal adaptation for Coastal Protection by Mangroves through Phylogenetic Adaptations

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Abstract:

Molecular phylogenetic has become a critical tool in understanding the genetic diversity and evolutionary relationships within mangrove species, which are complex plant communities found along tropical and subtropical coastlines. This study utilizes DNA barcoding to characterize mangrove diversity in India, particularly focusing on populations from the Sundarbans Delta. In the present research, we employed Bayesian inference, MEGA11 and MrBayes to analyse six DNA sequences from five genera, including *Volkmameria inermis, Avicennia alba, Suaeda maritima, Rhizosphora mucronata, Aegiceras corniculatum* and *Avicennia marina*. Notably, the study places all the 5 DNA sequence within the same monophyletic group and one species in another group. All mangrove genera and species could be satisfactorily discriminated using the concatenated *rbc*L + *mat*K loci. Additionally, the utility of markers *mat*K and *rbc*L for rapid species identification and biodiversity assessments is examined. The markers demonstrated high rates analysis success with *matK* providing the highest accuracy for species identification. This research underscores the importance of DNA barcodes in managing and conserving mangrove diversity and highlights the need for further development of barcode markers to fully resolve species and identify hybrids. The findings provide a basis for enhanced taxonomic clarity and conservation strategies in mangrove ecosystems.

Keywords: Molecular, diversity, markers, *mat*K, *rbc*L

Introduction

Mangroves are unique ecosystems that are found where the land and the sea converge. These distinctive coastal biomes are mostly found in the tropics and subtropics, where they develop dense, intertidal forests that are essential for maintaining biodiversity, protecting the coast, and sequestering carbon. Mangrove's capacity to survive in very salty, anoxic, and fluctuating intertidal zones, due to their specialized root systems and mechanisms for salt tolerance and water

conservation, underscores their evolutionary adaptations as well as their ecological resilience (Tomlinson, 1986; Chen et al., 2022). The evolutionary foundations of these adaptations have come under more and more scrutiny in recent research, which has illuminated the ways in which these plants prevent storm surges, reduce coastal erosion, and preserve water quality (Ellison, 2021; Kumar et al., 2023).

Numerous difficulties presented by the intertidal environment, including excessive salinity, abrupt tidal variations, and recurrent submergence, call for particular physiological and molecular adaptations. A variety of adaptations are seen in mangroves, including methods for withstanding salinity, tactics for conserving water, and unique root systems that stabilize coastal sediments and lessen the effects of waves (Chen et al., 2022). These plants have distinct gene expression patterns at the molecular level that support effective photosynthesis during stressful situations, salt detoxification, and osmotic adjustment (Zhao et al., 2023).

Taxonomically diverse and representing at least 20 unrelated plant families, mangroves exemplify convergent evolution, with phylogenetic analyses shedding light on the ways in which diverse clades of mangrove species have adapted to changing environmental conditions (Ellison et al., 1999; Smith et al., 2022). Despite their taxonomic complexity, mangroves share common physiological and molecular strategies for coping with intertidal stresses (Dassanayake et al., 2009; Wang et al., 2024). Comparative genomic investigations revealed convergent evolution features amongst distantly related mangrove species, suggesting that identical functional qualities might evolve as a result of similar environmental stressors (Smith et al., 2022). Furthermore, historical biogeographic patterns of mangroves have been revealed by phylogenetic studies, providing insight into how previous climatic changes impacted their worldwide distribution and regional adaptations (Wang et al., 2024).

Mangroves, located at the interface of land and sea, play a crucial role in coastal protection by mitigating erosion, buffering against storm surges, and providing habitats for diverse flora and fauna. Their adaptability to the harsh intertidal environment is a testament to their remarkable resilience. However, the molecular mechanisms underlying these adaptations remain largely unexplored, representing a gap in our understanding of mangrove biology (Zhao et al., 2023). Phylogenetic adaptations, shaped by evolutionary processes over millions of years, hold the key to understanding the genetic basis of intertidal adaptation in mangroves. By tracing the evolutionary history of mangrove species and comparing genomic sequences, researchers can uncover genetic signatures of adaptation to intertidal environments. Phylogenetic analyses allow for the identification of conserved genes, gene families, and regulatory elements that have been subject to natural selection for intertidal adaptation. To explore the molecular mechanisms underlying mangrove adaptation, comparative genomic studies are essential. These studies can unravel genetic signatures of adaptation to intertidal environments, identify conserved genes subject to natural selection, and elucidate regulatory elements driving adaptation (He et al., 2015; Yang et al., 2015a). Additionally, DNA barcoding and phylo-geographic estimation offer valuable tools for understanding the evolutionary relationships among mangrove species and revealing hidden richness in mangrove ecosystems (Plaziat et al., 2001). By integrating taxonomic, ecological, and genomic perspectives, we can enhance our understanding of mangrove evolution and adaptation, informing conservation strategies aimed at preserving these critical coastal ecosystems in the face of environmental change and anthropogenic disturbances.

This present work aims to synthesize current knowledge on the molecular mechanisms of intertidal adaptation in mangroves, focusing on phylogenetic adaptations. Understanding the genetic basis of mangrove resilience to intertidal stressors enhances our fundamental knowledge of plant adaptation and informs conservation strategies aimed at preserving mangrove ecosystems in the face of climate change and anthropogenic disturbances. DNA barcoding and phylo-geographic estimation of mangroves from India were rarely conducted. The purpose of this work is to use DNA barcoding to clarify the evolutionary links between several mangrove species. The study revealed the hidden richness of

mangroves in the Sundarbans Delta using DNA sequence data from the chloroplast region (*mat*K and *rbc*L genes). This study utilized DNA barcoding to explore the evolutionary relationships among various mangrove species in India, revealing the hidden richness of mangroves in the Sundarbans Delta, using Chloroplast region (*mat*K and *rbc*L gene) DNA sequence data.

Background:

Mangroves stand as nature's frontline defence against coastal hazards, showcasing remarkable adaptability to the harsh intertidal environment. Alongi (2008) underlines their importance in building resilience against coastal hazards and responding to global climate change. The ability of mangroves to adapt to acute and chronic disruptions is a topic of great interest in research. Studies have shown that mangroves exhibit remarkable resilience over evolutionary timescales, with soil accretion rates keeping pace with sea-level rise (Alongi, 2008). This resilience is demonstrated by their ability to recover from natural disasters such as storms and hurricanes (Alongi, 2008). Delving deeper into the dynamics of mangrove ecosystems, Duke, Ball, and Ellison (1998) shed light on the factors influencing biodiversity and distributional gradients in mangroves. They highlight the role of dispersal mechanisms, establishment success, and growth responses in shaping the distribution of mangrove species along environmental gradients.

Kathiresan and Bingham (2001) provide a comprehensive overview of mangrove biology and ecosystems, emphasizing their ecological importance. Mangroves, with their unique morphological and physiological adaptations, thrive in conditions of high salinity, extreme tides, and anaerobic soils. These adaptations enable mangroves to create unique ecological environments that support rich biodiversity and serve as crucial nursery habitats for various marine species. Understanding the environmental variables that influence mangrove establishment and early development is critical for successful conservation and management. Krauss et al. (2008) investigated these drivers, providing insights into the intricate interplay of biotic and abiotic factors that affect mangrove seedling survival and growth.

Feng et al. (2020) investigate the physiological and molecular aspects of mangrove salt tolerance, revealing intricate mechanisms that enable mangroves to thrive in saline environments. Their study on *Sonneratia alba* sheds light on the genetic basis of salt adaptation in mangroves, providing valuable insights for future research and conservation efforts. Osland et al. (2017) examine mangrove expansion and contraction patterns in response to climate extremes, highlighting the role of land-ocean temperature gradients in shaping mangrove distributions. Their findings underscore the importance of understanding the ecological implications of changing climate conditions on mangrove ecosystems. Saenger, Hegerl, and Davie (1983) provide a global perspective on the status of mangrove ecosystems, emphasizing their ecological significance and vulnerability to anthropogenic pressures. Their work underscores the urgent need for conservation and management efforts to safeguard these valuable coastal habitats. Tomlinson (2016) offers a comprehensive botanical exploration of mangroves, elucidating their morphological and anatomical adaptations to intertidal conditions. His work provides valuable insights into the structural diversity of mangroves and their evolutionary adaptations.

Bridging the gap between ecological theory and practical management strategies, Twilley and Rivera- Monroy (2005) develop simulation models to assess the performance of mangrove wetlands. Their work provides a framework for evaluating mangrove restoration projects and guiding conservation efforts. Wang, Vinocur, and Altman (2003) delve into plant responses to environmental stressors, providing insights into the genetic basis of stress tolerance mechanisms in mangroves. Their research lays the groundwork for future studies aimed at enhancing the resilience of mangroves to climate change and anthropogenic disturbances.

The integration of DNA barcoding techniques in mangrove research represents a significant step forward in our understanding and conservation efforts for these vital ecosystems. Saddhe et al. (2016) highlight the importance of

accurate species identification in mangrove conservation, especially considering the lack of taxonomic expertise and the threat of biodiversity loss. By employing DNA barcoding using plastid markers *rbc*L and *mat*K, they successfully assessed 14 mangrove species from Goa, India. Their study not only demonstrated high PCR amplification success rates but also provided valuable insights into the effectiveness of different barcode markers for species identification. Similarly, Rani et al. (2021) emphasizes the need for DNA barcode- based phylogenetic to characterize mangrove diversity in India. Their study, focusing on the Sundarbans Delta and Kerala, utilized the ITS locus of the nuclear genome to generate DNA sequences and assess phylogenetic relationships. The incorporation of DNA barcoding in mangrove research holds promise for enhancing our ability to accurately identify species, understand their evolutionary affinities, and ultimately guide conservation efforts. Each of these studies contributes to our understanding of mangrove adaptation for coastal protection, enriching our knowledge of these vital ecosystems and informing conservation and management efforts. And future work in this area will likely involve the evaluation of additional barcode markers to achieve complete resolution of mangrove species and identify putative hybrids, further advancing our knowledge and conservation strategies for these unique ecosystems.

Purpose of work: The aim of the study is to investigate phylogenetic adaptations in mangrove ecosystems to understand plant adaptability and guide conservation strategies amidst climate change and human disturbances.

Methodology

We used DNA sequence data of 6 collected true and associated Mangroves species from the *mat*K (Maturase K) and *rbc*L (Ribulose bisphosphate carboxylase) region for revealing the hidden biodiversity of mangroves from Sundarbans Delta.

1. **Nucleotide sequences were retrieved from NCBI Blast database website**: In order to confirm their identification with further information, nucleotide sequences were taken from the NCBI sequence database. To find conserved sites, variable sites, parsimony informative sites, singleton sites, transition/transversion ratio, and nucleotide pair frequencies, multiple sequence alignments were carried out using the MUSCLE programme and MEGA11 software (Tamura et al., 2011). A programme called Seqstat was used to code the GC content and indels.
2. **Selection of the nucleotide substitution model:** A statistical technique for phylogenetic inference based on the posterior probability of a phylogenetic tree is called Bayesian phylogeny (Huelsenbeck and Ronquist, 2001). According to the likelihood of seeing data conditioned on model parameters, it calculates the highest likelihood of an observed substitution. jModelTest 2.1.7 v20150530 was used to determine which nucleotide substitution model was optimal for Bayesian analysis. Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were then used to determine maximum likelihood analysis (Aho, Derryberry, and Peterson, 2014).

3. **Bayesian Inference tree analysis of targeted regions:**

For two million generations, DNA sequences were aligned using MEGA11 software and examined with MRBAYES v.3.1.2 (Ronquist and Huelsenbeck, 2003). Branch lengths were proportional to the number of nucleotide substitutions per site, and posterior probabilities were provided at nodes. Using the Figtree programme v1.4.0, the phylogenetic tree was shown (Rambaut, 2016).

Results

Six species, five genera, and five families of true and related mangroves were selected. The nucleotide sequences were individually retrieved from NCBI sequence database to determine their identity with other sequence information available in nucleotide databases and NCBI BLAST was used to validate the species identity using sequenced data. The purpose of this study is to verify mangroves at the species level in an initial manner. The species that have been identified include *Volkameria inermis, Aegiceras corniculatum, Suaeda maritima, Rhizosphora mucronata, Avicennia marina, and Avicennia alba*. Using MEGA11 software, phylogenetic analysis was performed on 23 DNA sequences that were obtained from NCBI and 6 sequences that were created specifically for this purpose. The analysis produced a well-resolved phylogram.

Nucleotide sequence analysis of *mat*K and *rbc*L in retrieved Mangrove species: In each case, the amplification profiles revealed length of 0.9kb for *mat*K region and 0.7kb for *rbc*L region. While the amplicons representing the *mat*K region showed an average G+C content of 32.5%, those representing the *rbc*L region showed an average G+C content of 44.37%. Nucleotide sequences of 917 bases, representing the *mat*K region from 6 species of mangrove studied in the present investigation, were aligned to construct a multiple alignment of the chloroplast genome's standard barcoding region. Clustal multiple alignment of nucleotide sequences of amplicons representing the *mat*K gene amplified in the present investigation revealed the presence of 3 indels. Sequence analysis revealed 48.63% conserved sites, 38.71% parsimony informative sites, 9.16% singleton sequence diversity, 47.87% variable sites and a transition/transversion ratio of 1.1. (Table 1). Likewise, nucleotide sequences of 685 bases, representing the *rbc*L region were aligned to construct a clustal multiple alignment. Clustal multiple alignment of nucleotide sequences of amplicons representing the *rbc*L gene from 6 species of Mangrove studied revealed 0 indels with 72.7% conserved sites, 19.70% variable sites, 15.47% parsimony informative sites, 3.94% singleton sequence diversity, and a transition/transversion ratio of 1.7 (Table 1). The best fitting nucleotide substitution model test was performed using MEGA version 6 (Tamura et al. 2013), and the model with highest BIC score = 6603.346 for *rbc*L and BIC score = 9280.708 for *mat*K was selected. The phylogenetic analysis was done with MEGA11 using Maximum likelihood (Huelsenbeck and Ronquist 2001).

Intra and interspecific divergence analysis between *mat*K and *rbc*L in Chenopodium

According to the study, in six mangrove species barcoding demonstrated that the intraspecific variability of 0.24% in *rbc*L and 0.20% in *mat*K, and average interspecific differentiation of 0.35% and 0.9% in *rbc*L and *mat*K, respectively. The greatest average pairwise distances, 2.05 in *mat*K and 0.68 in *rbc*L, were found by intraspecific and interspecific analysis, respectively. Compared to the interspecific variation revealed by the *mat*K and *rbc*L genes, the intraspecific variation between mangroves accessions was negligible. For all the marker, the intra- and interspecific divergence variability is displayed in Table 2.

Bayesian Inference analysis of sequences of *mat*K and *rbc*L

For Bayesian Inference analysis, amplified sequences of *mat*K and *rbc*L gene were aligned using the ClustalX program with the DNA sequences retrieved from NCBI databases following default settings for multiple alignment parameters at gap penalty 15, floating penalty 6.66, and transition DNA weight of 0.5. The aligned sequences were exported using nexus format (.nxs) for further use in phylogenetic analysis. A Bayesian inference (BI) tree for the *mat*K

gene constructed using the MRBAYES plugin (Huelsenbeck and Ronquist, 2001) and employed GTR+G+I substitution model for *mat*K region and *rbcL* gene employed T92+G substitution model (Fig 2). The study used a Markov chain to run for 2,00,000 generations, with every 1000th tree sampled. The remaining trees were combined to find the posterior probability estimate of phylogeny.

The maximum likelihood (ML) phylogenetic tree generated from the alignment of the nucleotide sequences of *rbc*L gene resolved 6 species into two major clusters divided in sub-clades. Where, Cluster-I comprised of 5 species namely *Rhizophora mucronata, Aegiceras comiculatum, Suaeda maritima, Volkaria inermis and Avicennia alba* which showed greater closeness in phylogenetic relationship, however cluster-II comprised of 1 species namely *Avicennia marina* which showed greater closeness in phylogenetic relationship respectively. While cluster I of 5 species form sub-clades of same speicies as *Suaeda maritima* voucher Gillespie 8616 for clade with *Suaeda maritima* voucher Gillespie et al 7570, *Suaeda maritima* voucher Ovenden 2241A CAN and *Suaeda maritima* respectively. While cluster –II comprised of *Avicennia marina* which shows great closeness between accessions *viz Avicennia marina* MNG35, *Avicennia marina* EDNA15-0042671, *Avicennia marina* isolate EDNA15-0042653 and *Avicennia marina* 4 (Fig 1). Likewise, the 50% majority-rule consensus tree constructed after the alignment matrix of nucleotide sequences of the 0.9kb *mat*K gene resolved the accessions into 2 clusters with posterior probability values of 99.97, wherein each cluster had of several subclusters. While cluster-I comprised of accessions belonging to 5 species viz *Rhizophora mucronata, Aegiceras comiculatum, Suaeda maritima, Avicennia marina and Avicennia alba* which showed closeness with each other. Cluster-II comprised of accessions belonging to only one species *Volkaria inermis* which shows great closeness between accessions *viz Volkaria inermis* isolate ine8, *Volkaria inermis* isolate ine10, *Volkaria inermis* isolate ine9 *and Volkaria inermis* P4190 which shows great closeness among themselves (Fig 1).

In order to evaluate the viability of the *rbc*L and *mat*K gene areas in mangrove species as DNA barcode markers for phylogenetic analysis, a comparison of these regions was conducted. While the *mat*K area revealed a lower degree of conservation (48.63% conserved sites) and more variability (three indels), the *rbc*L region had a high number of conserved sites (72.7%) and a transition/transversion ratio of 1.6. The percentage of parsimony informative sites in the *mat*K region was higher (38.71%), indicating that it might offer better phylogenetic resolution within mangrove species. Its lower overall conservation, however, indicates that for a thorough phylogenetic study, a mixed strategy utilising both markers could be best. The modest GC content of 38.28% and well-resolved phylogram demonstrate the usefulness of these markers in the molecular phylogenetics of mangroves. To increase phylogenetic resolution and support conservation efforts, more research on new markers is required.

Discussion

Mangroves, originating from diverse plant families, have developed shared adaptive mechanisms to thrive in harsh intertidal habitats, suggesting potential evolutionary convergence in their "mangrove lifestyle". However, the genetic underpinnings of this convergence remain debated. Studies comparing transcriptomes of mangroves with terrestrial species have yielded valuable insights. Dassanayake et al. (2009) observed similarities in transcriptome profiles between mangrove and terrestrial plants, hinting at convergent evolution at the transcriptome level. Conversely, others reported a lack of mangrove-specific genetic components (Chen et al., 2011; Yang et al., 2015a,b).

In our investigation, we examined the nucleotide sequences of the *mat*K and *rbc*L regions retrieved from mangrove species, which offers valuable insights into their genetic diversity and phylogenetic relationships. The amplification profiles consistently revealed lengths of 0.9kb for the *mat*K region and 0.7kb for the *rbc*L region. Notably, the *mat*K region exhibited a lower average G+C content of 32.5%, while the *rbc*L region showed a higher average G+C content of 44.37% which is similar to the findings of Guo et al., (2017) where they studied the transcriptome of four

mangrove species using next-generation sequencing technology. These differences in G+C content may reflect variations in sequence composition and evolutionary constraints between the two regions. Furthermore, pairwise distance analysis indicated a relatively low rate of nucleic acid substitution at both loci.

Based on research from literature and field observations, Barik and Chowdhury updated the list of genuine mangroves from the Indian Sundarbans delta in 2014, adding *A. alba Blume, A. marina vierh*., and *A. officinalis* L. Using Bayesian inference, maximum likelihood, and maximum parsimony techniques, Li et al. (2016) rebuilt the phylogenetic tree and proposed three main subclades: *A. marina* complex, *A. rumphiana* and *A. alba*, and *A. officinalis* and *A. integra*. Although they do not cluster with *A. rumphiana* and *A. alba*, the *A. marina* complex clade and the *A. officinalis/A. integra* subclade form a sister clade. Additionally, they examined the physical traits of the flowers, finding that *A. officinalis* maintains the 1.0 mm style length and ancestral stigma location, whereas *A. alba/A. rumphiana* lineage and *A.marina* complex display independent degeneration. Similarly, in current research upon aligning the nucleotide sequences of the *mat*K and *rbc*L regions, several important findings emerged. The analysis of the *mat*K region revealed the presence of three indels, suggesting sequence variation among the studied mangrove species. Additionally, the sequence analysis demonstrated a relatively high proportion of conserved sites (48.63%), parsimony informative sites (38.71%), and variable sites (47.87%) in the *mat*K region. In contrast, the *rbc*L region showed no indels and exhibited a higher proportion of conserved sites (72.7%), albeit with fewer variable sites (19.70%) and parsimony informative sites (15.47%).

Intra and interspecific divergence analysis indicated that the *mat*K region exhibited greater intraspecific and interspecific diversity compared to the *rbc*L region. This suggests that the *mat*K gene may be more informative for distinguishing between closely related mangrove species. Furthermore, the Bayesian Inference analysis of the sequences revealed distinct clusters and subclusters, providing insights into the phylogenetic relationships among the studied mangrove species.

Of note, species with multiple individuals forming a monophyletic clade in ML trees with a bootstrap value above 60% were considered as successful adaptations, following the criteria established by Kress et al. (2010). This criterion allowed for the identification of successful adaptations within the studied mangrove species. However, Saddhe et al. (2016) found a monophyletic relationship between Avicennia and Acanthaceae in Goa region, West Coast of India. They used markers psbK-psbI, rpoC1, and atpF-atpH to resolve Avicennia genera, revealing *A. marina* and *A. officinalis/A. integra* belong to the sister clade, while *A. alba* is genetically distinct. Moreover, this study represents an attempt at performing DNA barcoding-based diversity assessment of mangroves from the Sundarbans delta using chloroplast region markers through the *rbc*L and *mat*K genes. This pioneering effort builds upon previous research efforts (Lakshmi et al., 1997, 2000) and contributes to our understanding of mangrove diversity in this ecologically significant region. In the current study, the results of pairwise distance analysis indicate a relatively low rate of nucleic acid substitution at this locus.

The study shows that factors that contribute to the barrier to gene flow in mangrove species include marine currents, environmental adaptations, selection pressure, and changing climatic circumstances. Barriers to gene flow and population fragmentation are also caused by cyclones, global warming trends, and sea level rise. Mangrove species, including flowering plants, are classified as belonging to the taxonomic category Pentatetalae or Tricolpales, while belonging to separate families and genera. For the first time, DNA barcodes from the chloroplastic area are produced by the study.

Overall, the comprehensive analysis of the *mat*K and *rbc*L regions provides valuable insights into the genetic diversity and phylogenetic relationships of mangrove species. These findings enhance our understanding of mangrove evolution

and adaptation and have implications for conservation and management strategies aimed at preserving these vital coastal ecosystems.

Conclusion

In conclusion, our finding advocate that by comparing the *rbc*L and *mat*K gene areas in mangrove species as DNA barcode markers for phylogenetic analysis found that *mat*K had a lower conservation degree and more variability. The *rbc*L region had a high number of conserved sites (72.7%) and a transition/transversion ratio of 1.6. The *mat*K region had a higher percentage of parsimony informative sites (38.71%), suggesting better phylogenetic resolution within mangrove species. However, its lower conservation suggests a mixed strategy using both markers for a thorough study. The study also found that *mat*K is a useful DNA barcode marker for mangrove species, but a dual-marker method including both genes is recommended for more precise evolutionary connections.

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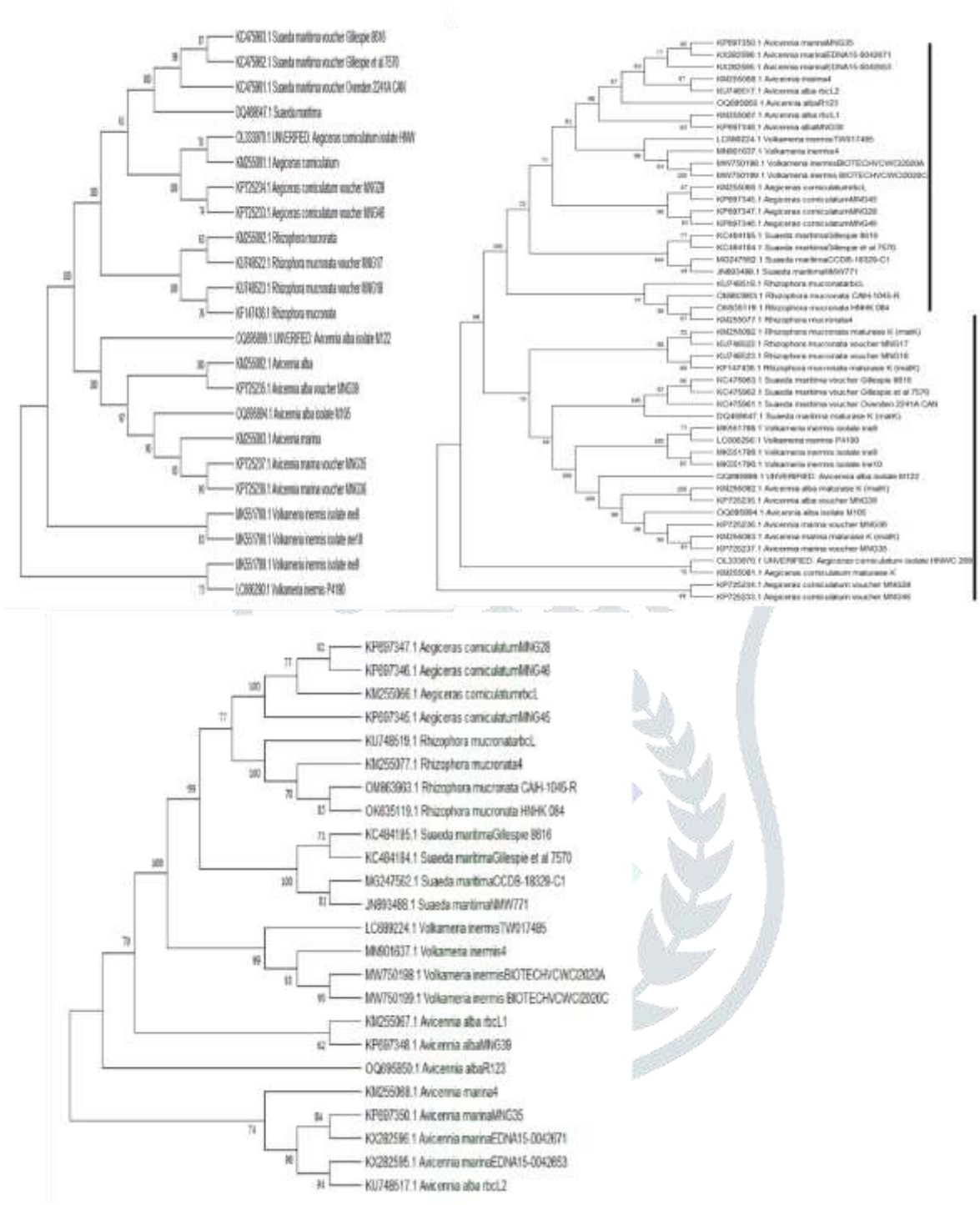

Figure 1: Maximum likelihood (ML) (using bootstrap value of 1000 replicates). **a** *rbc*L, **b** *mat*K, and **c** *rbc*L + *mat*K concatenated ML trees.

Figure 2: Maximum likelihood nucleotide substituition model showing best fit model for AIC and BIC parameters.

Table 1: Indels, sequence statistics and nucleotide pair frequencies analysis of sequences representing *mat*K and rbcl region of different accessions investigated in the present study. Nt: Nucleotide, CS: Conserved sites, VS: Variable site, PIS: Parsimony informative sites, SS: Singleton sites, ii: Identical pairs, si: transitional pairs, sv: transversional pairs, R: si/sv.

Sl.no	Taxa	Sequence statistics									Nucletide paired Frequencies				
		Length (Nt)	indels	% divergence	G+C (%)	CS (%)	VS (%)	PIS (%)	SS (%)	CpG (100 coverage)	CpG	ii	si	sv	R
1	*rbc*L	685	0	1.37	44.37	498 (72.7)	135 (19.70)	106 (15.47)	27 (3.94)	448	71	503	25	15	1.7
2	*mat*K	917	3	3.9	32.5	446 (48.63)	439 (47.87)	355 (38.71)	84 (9.16)	667	51	618	75	66	1.1
3	*mat*K+*rbc*L	921	3	2.5	35.46	219 (23.78)	676 (73.39)	575 (62.43)	98 (10.64)	404	1	386	102	110	0.9

Table 2: Analysis of intraspecific and interspecific variation within and between species.

Genetic Divergence	***rbc*L**	***mat*K**
Intraspecific distance	0.24	0.20
Average interspecific distance	0.35	0.90
The greatest average pairwise distance	*0.68*	*2.05*