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RESEARCH ARTICLE

Phylogenetic Analysis of *Excoecaria Agallocha* Using 18S Nuclear Ribosomal Gene

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ABSTRACT

Mangroves are woody plants which form the dominant variation in tidal, saline wetlands along tropical and subtropical coasts. *Excoecaria agallocha* is a mangrove species in the genus Excoecaria of the family Euphorbiaceae. 18S rRNA gene is one of the most important molecular markers, used in diverse applications such as molecular phylogenetic analyses and biodiversity screening. In the present study 18S gene of *Excoecaria agallocha* was amplified and sequenced. 18S gene was found to be 1659 base pairs (bp) in length where as GC% found 50.8. The number of nucleotide found to be 401 A's, 378 C's, 464 G's, 416 T's, and 0 N's. Phylogenetic tree infer that *Excoecaria agallocha* is closely related to *Rhizophora stylosa* and *Xylocarpus granatum*. Along with these two species *Excoecaria agallocha* forming monophyletic group with other closely related species such as *Cerbera manghas, Kandelia candel, Bruguiera gymnorhiza* and *Nypa fruticans.*

KEYWORDS

Excoecaria agallocha, Phylogenetic, rRNA gene

INTRODUCTION

Mangroves as a source of marine natural products.[1] Mangroves are halophyte plants.[2] They exist in conditions of high salinity, extreme tides, strong winds, high temperature, muddy and anaerobic soils.[3] These extreme conditions enable mangroves to yield secondary metabolites as chemical defense for their lives. The secondary metabolites be consisted in mangroves are alkaloid, triterpenoid, saponin, fitosterol and poliphenol.

Excoecaria agallocha is a mangrove species in the genus Excoecaria of the family Euphorbiaceae. This species is common in mangrove swamps from India. This species should be treated with caution.

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The milky exudate produced by most plant parts can cause intense pain and temporary blindness if it gets into the eyes and painful blistering on other sensitive body parts. It is also poisonous to stock. The latex is extremely poisonous. Even dried and powdered leaves contain the poison which can kill fish very quickly.

The evolutionary history of a set of taxa is usually represented by a phylogenetic tree, and this model has greatly facilitated the discussion and testing of hypotheses. Phylogenetic networks should be employed when reticulate events such as hybridization, horizontal gene transfer, recombination, or gene duplication and loss are believed to be involved, and, even in the absence of such events, phylogenetic networks have a useful role to play. This article reviews the terminology used for phylogenetic networks and covers split networks and reticulate networks, how they are defined, and how they can be interpreted. The small subunit (SSU)

18S rRNA gene is one of the most frequently used genes in phylogenetic studies and an important marker for random target polymerase chain reaction (PCR) in environmental biodiversity screening.[4] In general, rRNA gene sequences are easy to access due to highly conserved flanking regions allowing for the use of universal primers.[4] Their repetitive arrangement within the genome provides excessive amounts of template DNA for PCR, even in smallest organisms. The 18S gene is part of the ribosomal functional core and is exposed to similar selective forces in all living beings. The 18S rRNA gene is one of the most important molecular markers, used in diverse applications such as molecular phylogenetic analyses and biodiversity screening.

MATERIALS AND METHOD

Collection of Plant Material

Excoecaria agallocha was collected from Vashi creek, Navi Mumbai and used for research. Other 18S gene sequences were retrieved from NCBI genbank. Name and their family of retrieved species are given in following table.

Table 1: Name of species names and its family downloaded from NCBI genbank

No	Species	Family	No	Species	Family
1	*Excoecaria agallocha*	Euphorbiaceae	11	*Bruguiera gymnorhiza*	Rhizophoraceae
2	*Sonneratia ovata*	Sonneratiaceae	12	*Kandelia candel*	Rhizophoraceae
3	*Rhizophora stylosa*	Rhizophoraceae	13	*Barringtonia racemosa*	Lecythidaceae
4	*Nypa fruticans*	Arecaceae	14	*Cerbera manghas*	Apocynaceae
5	*Laguncularia racemosa*	Combretaceae	15	*Xylocarpus granatum*	Meliaceae
6	*Avicennia marina*	Acanthaceae	16	*Scyphiphora hydrophyllacea*	Rubiaceae
7	*Ceriops tagal*	Rhizophoraceae	17	*Pemphis acidula*	Lythraceae
8	*Ceriantheopsis americana*	Cerianthidae	18	*Aegiceras corniculatum*	Myrsinaceae
9	*Heritiera littoralis*	Sterculaceae	19	*Haloaleurodiscus mangrovei*	Homobasidiomycetes
10	*Lumnitzera littorea*	Combretaceae	20	*Dolichandrone spathacea*	Begoniaceae

Isolation of Genomic DNA

Total genomic DNA was isolated from fresh leaves using a modified genomic DNA isolation protocol.[5] Plant material was collected during rainy season. 0.5-1 gm of leaf tissue was used to isolate genomic DNA. The DNA was then resuspended in TE buffer (10 mM: 1 mM) and stored at -20 °C

Quality and quantity of DNA was checked by loading 2µl genomic DNA and 3 µl of gel loading dye on 1.5% agarose gel. The amount of DNA obtained ranged from 20 µg to 60 µg.

PCR Amplification and DNA Sequencing

The entire 18S region of *Excoecaria agallocha* was PCR amplified in a thermal cycler (Eppendorf) using Forward primer (GTAGTCATATGCTTGTCTC) and Reverse primer (GAAACCTTGTTACGACTT). Reaction volume was 50 µL and contained, 1X Taq DNA Polymerase buffer (Bangalore genei, India), 1.5 mM $MgCl_2$ (Bangalore genei, India), 200 µmolar each deoxynucleotide triphosphate (Bangalore genei, India), 10 pmol oligonucleotide primers (Bangalore genei, India), 1.0 unit of Taq DNA polymerase (Bangalore genei, India), and ~25–60 ng of genomic DNA. PCR was performed in a thermal cycler (Eppendorf) and consisted of initial denaturation of 5 min at 94°C, 35 cycles of 1 min at 94°C for template denaturation, 1 min at 50°C for primer annealing, 1 min at 72°C for primer extension, followed by a final extension of 5 min at 72°C. PCR products were subsequently visualized on a 1.5% agarose gel. Purified products were sequenced, using the same conditions as the PCR. 18S sequences of *Excoecaria agallocha* was obtained using both primers and sequencing was carried out on ABI Sequencer (Chromous Biotech, Bangalore) with minor manual adjustments.

RESULTS AND DISCUSSION

The length of 18S regions and GC % of were calculated by using online bioinformatics tools. The length of 18S gene found to be 1659 base pairs (bp) where as GC% found 50.8. The number of nucleotide found to be 401 A's, 378 C's, 464 G's, 416 T's, and 0 N's. Sequence submitted to NCBI genbank with submission ID is: 1580286.

Sequence Alignment and Phylogenetic Tree

All 18S gene sequences of mangrove were retrieved from NCBI genbank and aligned with 18S region of *Excoecaria agallocha* using Clustal computer program with Gap Open Penalty 15 and Gap Extension Penalty 6.66.[6]

Phylogenetic tree infer that *Excoecaria agallocha* is closely related to *Rhizophorastylosa* and *Xylocarpusgranatum.* Along with these two species *Excoecaria agallocha* forming monophyletic group with other closely related species such as *Cerbera manghas*, *Kandelia candel*, *Bruguiera gymnorhiza* and *Nypa fruticans.* Whereas *Sonneratia ovata, Laguncularia racemosa, Pemphis acidula, Lumnitzera littorea* are forning group of closely related species. *Avicennia marina, Dolichandrone spathacea, Scyphiphora hydrophyllacea, Cerbera manghas* and *Scyphiphora hydrophyllacea* found to be closely related and are in one group. *Haloaleurodiscus mangrovei* and *Ceriantheopsis americana* are distantly related from *Excoecaria agallocha.*

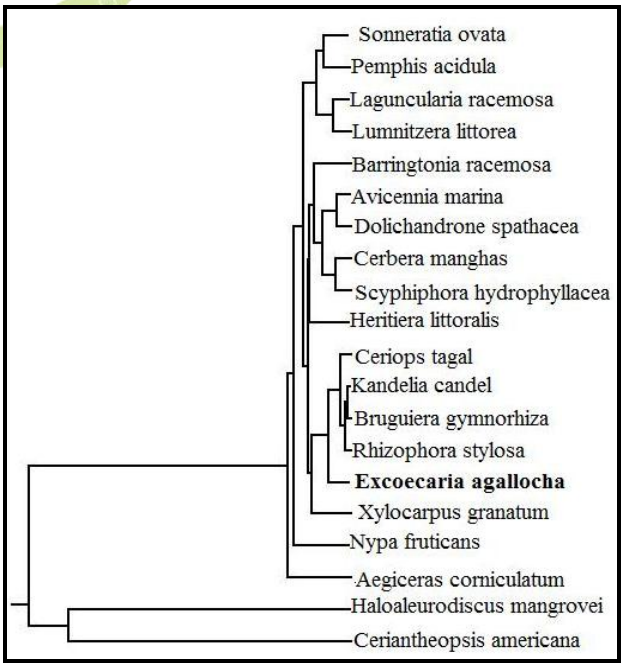

Figure 1: Phylogenetic tree in mangroves

Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants.

CONCLUSION

Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants. 18S gene is one of the useful phylogenetic marker which one can use in understanding phylogenetics.

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