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Phylogenetic Studies of Rudraksha; *Elaeocarpus* spp.

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ABSTRACT: *Elaeocarpus* is a diverse genus within the family Elaeocarpaceae. There is wide distribution of *Elaeocarpus* in the world among the tropical and subtropical climatic zones. In India, rudraksha (*Elaeocarpus sphaericus*) has important medicinal and religious values and its history dates back to ancient times. However, the evolutionary relationship of rudraksha with other species of *Elaeocarpus* is not much explored specially at the molecular and phylogenetic level. The present study establishes evolutionary relationship between rudraksha and other species of *Elaeocarpus* through phylogenetic algorithms like neighbor joining and maximum likelihood. Thirty species of *Elaeocarpus* found in the Indo-Australian region were grouped into clusters based on the rDNA and ITS sequence based phylogenetic analysis. This studies paves a way for further studies on evolutionary history of rudraksha with respect to other species of *Elaeocarpus* and their geographical distribution.

Keywords: Rudraksha, *Elaeocarpus*, Phylogenetics, Neighbor Joining, Maximum Likelihood.

I. INTRODUCTION

The family Elaeocarpaceae is found in the subtropical regions with exception of some genera which are found in the temperate zone. It is present in all continents except Africa and North America. *Elaeocarpus* is the largest genus of this family with more than 350 species distributed around the globe including India, Southeast Asia, Australia, Fiji, New Zealand, Japan, China, Madagascar and Hawaii. In India, *Elaeocarpus sphaericus* (syn. *Elaeocarpus ganitrus*) is a prevalent species; which is commonly known as "rudraksha". It is a large evergreen tree with broad leaves and is mostly found in subtropical and tropical areas [1].

Apart from India, the rudraksha tree is mainly found in Philippines, Manila, Australia, Myanmar, Bangladesh, Bhutan and Nepal. The rudraksha tree has medicinal and religious values especially in India. Parts of rudraksha tree are used as medicines for treatment of epilepsy, stress, depression, palpitation, nerve pain, migraine, arthritis hypertension, anxiety, liver diseases and asthma. *E. sphaericus* is also reported to have antibacterial and antioxidant properties [2]. The dried herbal fruit of rudraksha tree (Fig. 1) has been reported to have positive effects on nervous system and heart in ayurvedic medicine [3, 4].

Fig. 1. The rudraksha plant (A) and fruits and seeds of rudraksha (B).

Apart from Indian subcontinent, species of *Elaeocarpus* including *E. sphaericus* are also prevalent in the Australian tropical and subtropical rainforest regions. In Australia, the genus *Elaeocarpus* is most diverse in the regional ecosystem in Queensland with a recognizable feature of 30% in the Wet Tropics Bioregion [5]. The origin of rudraksha in particular has been stated to be in the mountains of Himalaya [6]; however a large number of species of *Elaeocarpus* is distributed all over the world with considerable morphological, physiological and structural similarities. This study aims to establish evolutionary relationship between 30 different species of *Elaeocarpus* found in the Indo-Australian region with the help of phylogenetics and bioinformatic tools.

II. MATERIALS AND METHODS

A. ITS sequence retrieval from NCBI databases

The sequences of internal transcribed spacer 1 (partial), 5.8s ribosomal RNA gene and internal transcribed spacer 2 were retrieved from NCBI Genbank database. The NCBI portal and BLAST servers were used for this purpose. The retrieved sequences were arranged in a text file in FASTA format for further analysis. These sequences were retrieved from 30 species of *Elaecarpus* including rudraksha (*Elaeocarpus sphaericus*) found in the Indo-Australian region. The list of sequences retrieved from Genbank is given in Table 1.

Table 1: List of nucleotide sequences retrieved from Genbank (NCBI).

Sr. No.	Accession Number	Species
1.	DQ448690.1	*Elaeocarpus ferruginiflorus*
2.	KJ675685.1	*Elaeocarpus. ford 4312*
3.	KJ675684.1	*Elaeocarpus pulchellus*
4.	KJ675683.1	*Elaeocarpus ptilanthus*
5.	KJ675682.1	*Elaeocarpus polydactylus*
6.	KJ675680.1	*Elaeocarpus hylobroma*
7.	KJ675679.1	*Elaeocarpus sphaericus*
8.	KJ675670.1	*Elaeocarpus thelmae*
9.	KJ675668.1	*Elaeocarpus baba 443*
10.	KJ675667.1	*Elaeocarpus sylvestris*
11.	KJ675666.1	*Elaeocarpus stipularis*
12.	KJ675665.1	*Elaeocarpus stellaris*
13.	KJ675664.1	*Elaeocarpus speciosus*
14.	KJ675663.1	*Elaeocarpus sedentarius*
15.	KJ675661.1	*Elaeocarpus obovatus*
16.	KJ675659.1	*Elaeocarpus nouhuysii*
17.	KJ675657.1	*Elaeocarpus johnsonii*
18.	KJ675654.1	*Elaeocarpus glaber*
19.	KJ675656.1	*Elaeocarpus hookerianus*
20.	KJ675653.1	*Elaeocarpus dongnaiensis*
21.	KJ675655.1	*Elaeocarpus grandis*
22.	KJ675652.1	*Elaeocarpus coorangooloo*
23.	KJ675651.1	*Elaeocarpus brachypodus*
24.	KJ675650.1	*Elaeocarpus bifidus*
25.	KJ675649.1	*Elaeocarpus bancroftii*
26.	KJ675648.1	*Elaeocarpus arnhemicus*
27.	KJ675646.1	*Elaeocarpus angustifolius*
28.	KJ675644.1	*Elaeocarpus alaternoides*
29.	KJ675681.1	*Elaeocarpus largiflorens* subsp. *largiflorens*
30.	KJ675658.1	*Elaeocarpus largiflorens* subsp. *retinervis*

B. Phylogenetic analysis

ITS sequences obtained from the Genbank database were aligned with ClustalW2 [7]. Phylogenetic analysis was done using MEGA7 [8] taking ITS sequences retrieved from thirty isolates of Indo-Australian origin to understand evolutionary relationship of rudraksha with other species of *Elaeocarpus*.

Neighbor joining method was used to infer evolutionary history [9]. The optimal tree with the sum of branch length = 24.48109160 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [10]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [11] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 469 positions in the final dataset.

The evolutionary history was also inferred by using the Maximum Likelihood method based on the Tamura-Nei model [12]. The bootstrap consensus tree inferred from 500 replicates [10] were taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) were shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 469 positions in the final dataset.

III. RESULTS AND DISCUSSION

In plant systematics, the nuclear and chloroplast genomic markers are most commonly used; while the mitochondrial genomic markers are seldom used due to the frequent structural rearrangements in the mitochondrial; genome [13]. The nuclear genome is the largest part of the genetic content of eukaryotic organisms [14]. The nuclear ribosomal DNA is one of the most frequently used part of nuclear genome for phylogenetic analysis [15]. There are three coding regions within the nuclear ribosomal DNA *viz.* 18s, 5.8s and 28s rDNA along with non-coding intergenic spacer (IGS), internal transcribed spacer (ITS) and external transcribed spacer (ETS). The reason for popularity of this region for phylogenetic analysis is availability of semi-universal primers and ubiquitous existence of its copies analysis [14, 15] Moreover, there is prevalence of mutations in the repetitive regions in this part of nuclear DNA along with existence of parallel homogenization, which makes it suitable for phylogenetic analysis [16].

In the present study, phylogenetic analysis of 30 species of *Elaeocarpus* including *E. sphaericus* (Rudraksha) found in the Indo-Australian region was done using sequences of internal transcribed spacer 1 (partial), 5.8s ribosomal RNA gene and internal transcribed spacer 2 regions. The neighbor joining method was used to infer evolutionary history, which grouped the 30 species into two major clusters; cluster I and cluster II (Fig. 2). Each cluster comprised of 15 species. In cluster I, *E. sphaericus* (rudraksha) showed similarities with a subcluster comprising of *E. dongnaiensis*, *E. sylvestris* and *E. glaber* with a bootstrap support of 48%. Within this subcluster, E. *dongnaiensis* showed a bootstrap support of 53% with *E. sylvestris* and *E. glaber*. Similarly, *E. sylvestris* and *E. dongnaiensis* showed evolutionary relationship with a bootstrap support of 62% in a different subcluster within cluster I. *E. speciosus* and *E. sedentarius* showed high degree of similarity with a bootstrap support of 99% within cluster I. *E. hookerianus* and *E. arnhemicus* showed 97% bootstrap support, while *E. coorangooloo* and *E. alaternoides* showed 80% bootstrap support.

Other members of cluster I include *E. nouhuysii*, *E. angustifolius*, *E. ferruginiflorus*, *E. brachypodus* and *E. obovatus*. In cluster II, *E. pulchellus* and *E. largiflorens* subsp. *retinervis* showed 100% bootstrap support; both of these showed 90% bootstrap support with *E., bancroftii*. Similarly, *E. ford* 4312 and *E. stellaris* showed 100% bootstrap support. High degree of similarity was also observed between *E. ptilanthus* and *E. johnsonii* showing 99% bootstrap support. Other members of cluster II include *E. hylobroma*, *E. thelmae*, *E. stipularis*, *E. largiflorens* subsp. *largiflorens, E. baba* 443*, E. bifidus, E. grandis* and *E. polydactylus*.

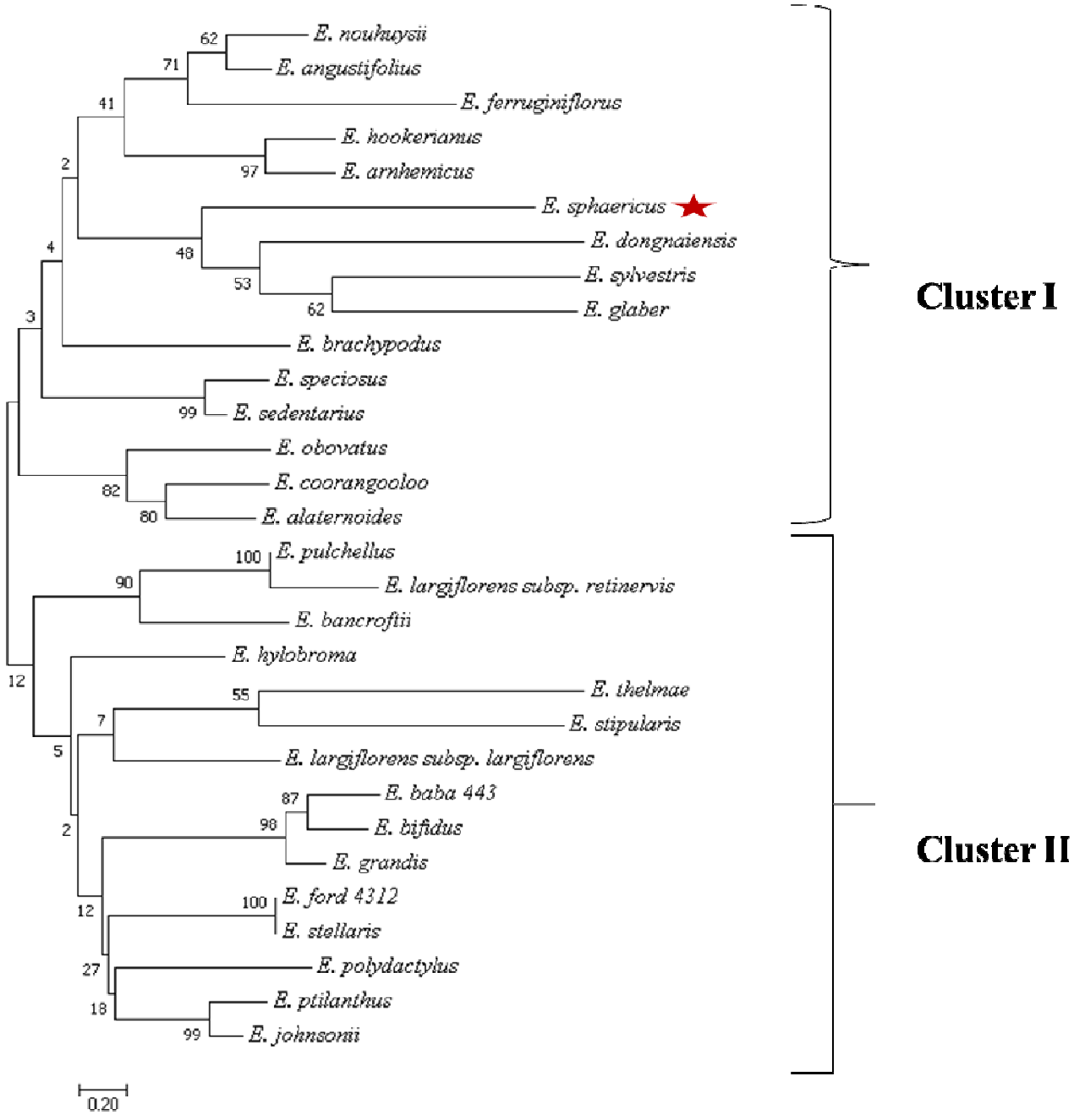

Fig. 2. Phylogenetic tree of different species of *Elaeocarpus* using neighbor joining method (MEGA 7).

Fig. 3 shows the phylogenetic tree obtained through maximum likelihood method. This tree also has two major clusters; cluster I and cluster II. However, 27 out of 30 species grouped into cluster one and further divided into different sub-clusters. Cluster II comprised of only 3 species. In this tree, *E. sphaericus* (rudraksha) grouped within cluster I showed 40% bootstrap support with *E. dongnaiensis*, *E. sylvestris* and *E. glaber*; on the other hand, it also showed 35% bootstrap support with *E. thelmae* and *E. stipularis*. Like the neighbor joining tree, *E. pulchellus* and *E. largiflorens* subsp. *retinervis* were found to be closely related with 92% bootstrap support. Similarly, *E. ford* 4312 and *E. stellaris* again showed 100% bootstrap support. Cluster II comprised of *E. brachypodus*, *E. hylobroma* and *E. largiflorens* subsp. *largiflorens.*

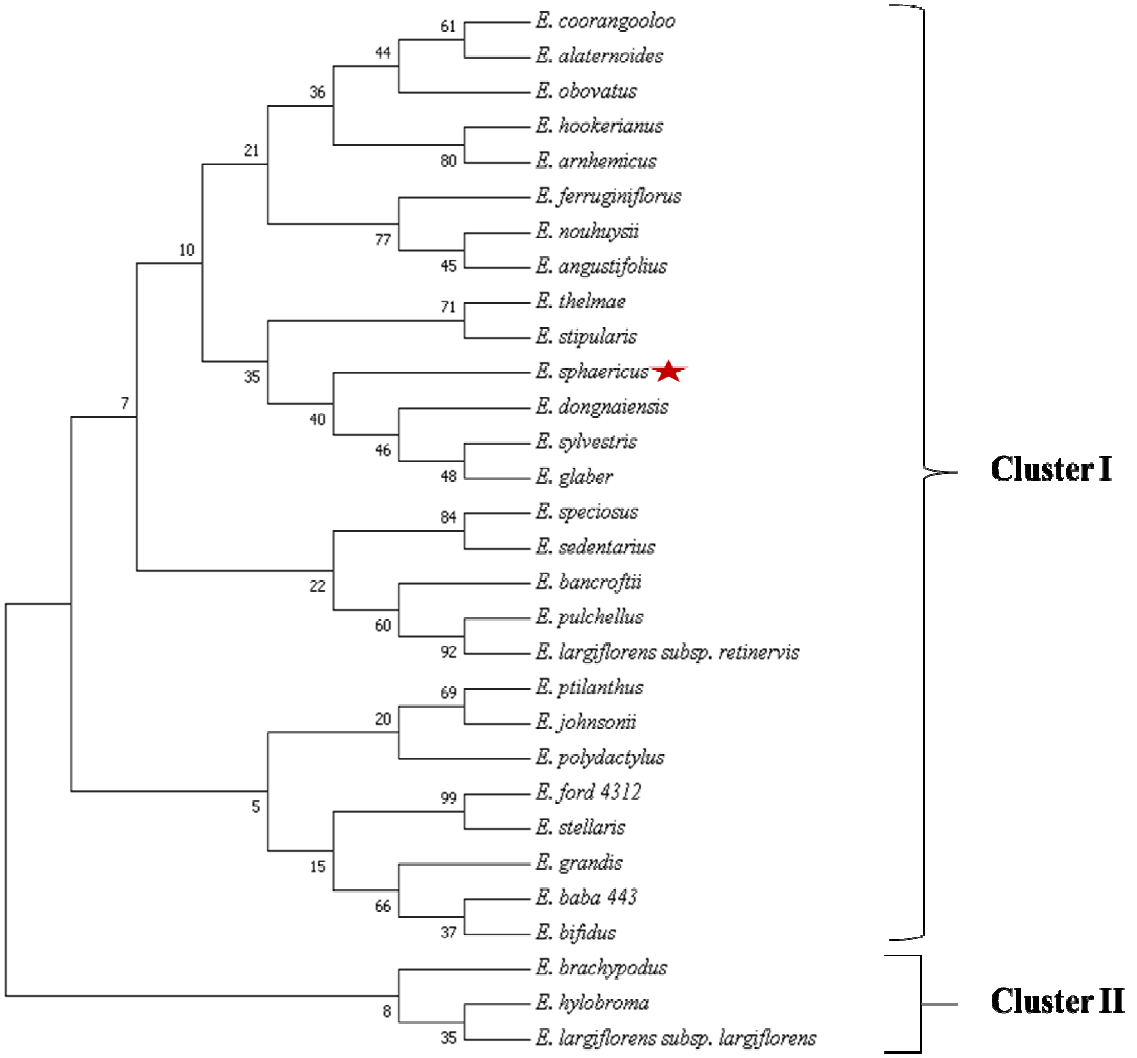

Fig. 3. Phylogenetic tree of different species of *Elaeocarpus* using maximum likelihood method (MEGA 7).

Evolutionary studies with respect to *Elaeocarpus* over the last decade has opened new doors for understanding new aspects of its evolutionary history which could not be achieved through morphological studies. Apparently, placement of family Elaeocarpaceae in Oxidales was strongly supported [17, 18]; which is contrary to previous morphology based classification of this family in Malvales. Although a general consensus about the number of species in the genus *Elaeocarpus* has been achieved (350-360); but the relationship between the different species has not been studied thoroughly specially at the molecular level.

IV. CONCLUSION

Rudraksha (*Elaeocarpus sphaericus*) is a medicinally important species found in India along with other countries of the world. It belongs to the diverse *Elaeocarpus* genus of the family Elaeocarpaceae. Morphological studies have established relationships between various species of this genus, however; studies at the molecular phylogenetic level to understand evolutionary history of rudrakksha and other species of the genus are limited. Our study demonstrates the evolutionary relationship between different species of *Elaeocarpus* including rudraksha found in the Indo-Australian region based on the conserved ribosomal and ITS region of nuclear DNA. This study can lay a plinth for further studies in molecular systematics of *Elaeocarpus* and understand their evolutionary history using different phylogenetic markers involving a larger number of species.

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