



## Phylogenetic Study on The Genus *Ficus* L. (Moraceae) in Kerala Using ITS Sequences as Molecular Marker

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### Abstract

A preliminary molecular phylogenetic study on *Ficus* species from Kerala is done using Internal Transcriber Spacer (ITS) sequences. 29 species of *Ficus* were studied here to establish their phylogenetic relationship. Sequences of the ITS 2 region were retrieved from NCBI, with reference to accession numbers from authentic specimens. The sequences were alligned and Neighbour Joining tree was prepared by MEGA 10 software. The dendrogram obtained well separated the *Ficus* species into two major groups. Most of the morphologically similar species showed a common lineage and were closely related. *F. caulocarpa* and *F. superba* were the closest species. *F. carica*, the type species of the genus stands distinct from other species in the dendrogram.

**Key Words:** *Ficus*, Figs, Phylogeny, ITS sequences, Kerala.

### Introduction

With a global distribution of around 750 species (Noor *et al.*, 2007; Chaudhary, 2012), *Ficus* L. (Moraceae), commonly referred as figs, is one among the most abundant and diverse group of angiosperm plants. They remain as a highly complex taxonomic group with respect to the morphological complexities and floral characters. Figs have the specific hypanthodium inflorescence, pollinated by

Agaonidae wasps exhibiting mutualism and co evolution (Priyadharsan 2000; Li *et al.*, 2012 a). Figs exhibit diversified habitat pattern including independent trees, hemi epiphytes, epiphytes and rarely some woody climbers. They fruit throughout the year, ensuring rich nutritional supply to many animals and birds feeding on the fig trees (Harrison, 2005). Thus, figs help to maintain an ecological balance in the ecosystem and act as a keystone species. According to the current status, 32 species of *Ficus* is reported from Kerala, belonging to 5 subgenera (Sasidharan, 2004).

DNA barcoding techniques aims to reveal the identity of plants that are morphologically indistinguishable. Various molecular markers are used to identify the specimens. Internal Transcribed Spacer (ITS) sequences are such molecular markers used in plant identification. ITS shows the most variable sites, greater intra- and inter-specific divergences and high species discrimination rate in the genus *Ficus* (Li *et al.*, 2012 b). Hence, ITS is the most suitable single locus DNA barcode in *Ficus*. The present study aims to provide a preliminary knowledge on the phylogeny of fig species in Kerala using ITS sequences.

### Materials and Methods

#### Selection of species and sequences



Out of the 32 species of *Ficus* present in Kerala, 28 species of *Ficus* were chosen for the study based on the availability of sequences in NCBI. Along with them, the type species of the genus *Ficus*, *Ficus carica* is also included in the study. Thus, a total of 29 species is studied. Relevant research articles were referred to get authentic accession number of the specimens. Accession numbers of the specimens were recorded from the research articles (Chantarasuwan *et al.*, 2015; Karthikeyan *et al.*, Li *et al.*, 2012 a & b; Ronsted *et al.*, 2008). The ITS sequences of the specimens were downloaded from NCBI, using the accession number.

### Phylogenetic Analysis

Sequence alignment of the ITS sequences retrieved from NCBI were done. Phylogenetic analysis was done using MEGA 10.1.7 software and Neighbour-joining (NJ) tree was prepared using the sequences. Divergence distance between the species is calculated to estimate the relatedness of the species.

### Result

Out of the 32 species of *Ficus* present in Kerala, ITS sequences of 28 species is available from NCBI. A Neighbour Joining tree is obtained by the phylogenetic analysis of 29 species of *Ficus*, using ITS 2 sequences and MEGA software, which shows distinct clustering of the species. Dendrogram obtained from the phylogenetic analysis is shown below in which the fig species were separated into two major groups. One with 17 species and the other with 12 species. Most of the morphologically similar species were placed in the adjacent branches. Dendrogram obtained from the phylogenetic analysis is shown in Fig 1.

### Discussion

The dendrogram obtained well separated all the species of figs. Morphologically similar species like *F. amplocarpa* and *F. guttata*, *F. dalhousiae* and *F. anamalayana* get separated supporting them as separate species. Out of the 32 species of figs studied, 16 of them shows close similarity and ended up in nodes with paired species. Divergence distance analysis shows that *F. caulocarpa* and *F. superba* were the closest species with a value very close to 0. With a value of 0.003403, *F. anamalayana* and *F. dalhousiae* were the second closest species. *F. virens* is the closest and *F. krishnae* is the most distant species of *F. carica*. *F. microcarpa* and *F. benjamina*, two morphologically similar species were separated with great reliability.

All the three species of figs with cauliflorous inflorescence were clustered together. None of the exotic species showed any particular divergence, from the native and indigenous species. The dendrogram also shows close similarity with the dendrogram based on syconium characters (Sreehari *Prs. Comm.*) and the current clustering separates the species similar to the natural classification into independent trees, epiphytes and trees with cauliflorous inflorescence. With evidence to the studies of Li *et al.*, 2012 a, which confirmed the ITS sequences as the most suitable molecular marker for *Ficus*, the current study also supports the reliability of ITS sequences in the phylogenetic studies of *Ficus*.

As almost 50 % of the species were close enough in their similarity, they were well separated into species. Hence, the current study implies that as ITS sequences could separate even the most morphologically similar species of *Ficus* into separate species, it is one among the molecular marker reliable for molecular studies in *Ficus*.

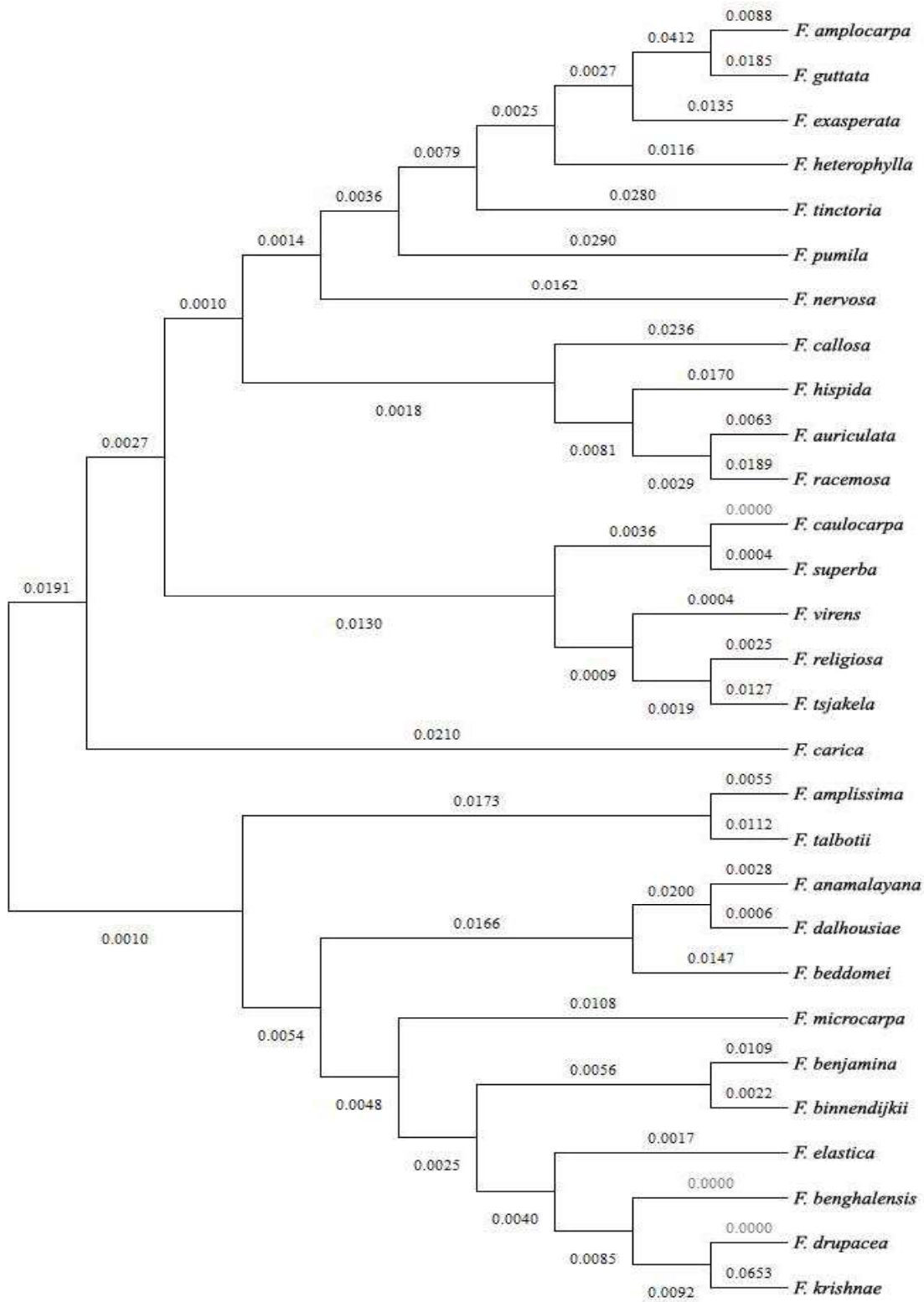


Fig 1: Dendrogram of the Neighbour-joining (NJ) tree based on ITS 2 region of Ficus species



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