Abstract

The present study aims at assigning several fungal samples collected from Pune region to the correct taxonomic groups on the basis of their morphological characters as well as DNA sequence similarities of their rDNA genes. A Total of 117 specimens were collected, 110 from, Pune region and 7 from Lausanne, Switzerland, from various Fabaceae and conifer tree species respectively.

The taxonomy of Hymenochaetaceae is primarily based on morphological characteristics, such as the shapes of basidiocarps and hymenophores, hyphal systems, and forms and sizes of basidiospores. The specimens were identified by studying macro and micro morphology. A dichotomous key was prepared. Based on morphological data, the specimens were assigned to 7 different genera (Fulvifomes, Fuscoporia, Fomitiporia, Phylloporia, Inocutis, Inonotus, Phellinus) of Hymenochaetaceae. Two specimens were assigned to each, Fomitopsis and Ganoderma (Polyporales). These specimens were used as an out-group for cladistic and phylogenetic analysis.

Cladistic approach was used to cluster the specimens and to study the relationships amongst them. For this, principal component analysis (MINITAB software) was carried out using 11 quantitative characters. It was observed that cladistic analysis did not lead to distinct clustering of all the samples.

The Internal transcribed spacer (ITS) region and 16S rDNA sequence diversity enables delimitation at interspecific or intraspecific level, while 25S rDNA helps to resolve the ambiguities at generic level. In the present study large subunit ribosomal DNA (25S rDNA), ITS region of nuclear rDNA and 16S rDNA or mitochondrial rDNA small subunit gene were used. Molecular analysis was carried out using sequence data of the collected samples and determining genetic relatedness of their sequences to those of other previously reported genera (from databases).
DNA isolation was carried out using CTAB and SDS method described in
literature with minor modifications. The various rDNA regions were amplified by
PCR and sequenced. The raw data was edited and the sequences were aligned
with Clustal W method using MEGA 4. A dendrogram was constructed using the
maximum parsimony algorithm in MEGA 4 (Tamura et al., 2007).

On the basis of morphological observations, samples collected from Pune
region included the genus *Fulvifomes* (represented by 91 specimens), which
constituted the largest represented group. Other represented genera included
*Fuscoporia* (2 specimens), *Inonotus* (5 specimens) and *Inocutis* (5 specimens).
All these samples represented tropical Hymenochaetaceae members. In addition,
some samples belonged to the genera *Phellinus* (2 specimens), *Phyllopora*,
*Fomitiporia* and *Fuscoporia* (1 specimen each). *Fomitopsis* (2 specimens) formed
out group. The latter were collected from Lausanne, Switzerland and represented
some temperate Hymenochaetaceae members. Co-clustering of the DNA
sequences of collected samples with those of previously identified (reported)
genera in PCA analysis and phylogenetic analysis was used to confirm the
identity of genera represented in the collected samples.

These studies showed that (a) the genus *Phellinus* was poorly represented
in the specimens collected from Fabaceae members in Pune region (b) *Fulvifomes*
was largely a tropical genus and differs from *Phellinus* in spore morphology and
presence of setae (c) The Hymenochaetaceae genera studied did not appear to
show host specificity. (d) Mitochondrial 16S rDNA – based clustering represented
the genetic relatedness between the genera better than rDNA sequences from 80S
ribosomal genes. (e) The temperate specimens of *Inocutis* did not co-segregate
with the tropical specimens, indicating different paths of genetic diversification
between tropical and temperate specimens of this genus.

**Keywords:** Classification, Hymenochaetaceae, ITS, 16S rDNA, 28S rDNA,
*Phellinus*, Phylogeny, *Inonotus*