



Establishment
27/11/1928

SNJB (Jain Gurukul's)

**K.K.H. Abad Arts, S.M.G. Lodha Commerce & S.P.H. Jain Science College
Neminagar, Chandwad-423101, Dist.-Nashik, Maharashtra**

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DST-FIST Funded (2018-19)

UGC-NSQF Courses (B.Voc. & CC)

Best College Award by Savitribai Phule Pune University (2015-16)

List of Documents: Botany M.Sc. II Field Project

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M. Sc II Field Project Students List

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20.	Sanap Pooja Kashinath
21.	Wagh Gayatri Balkrishna

BO 4.6 Research Methodology and Summer Training Report

I. Project (2 Credits)

Projects will be allotted in third semester and students will submit project work having introduction, review of literature, well defined material and methods, results and discussion, conclusions and references. The project should be presented at the end of fourth semester.

J. Review (1 Credit)

Based on review of literature on some advanced techniques in Botany and its presentation during practical examination

K. Summer Training (1 Credit)

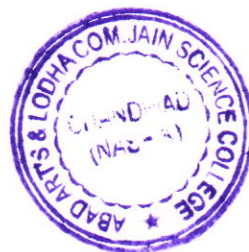
1. Report submission based on one summer training in research institutes/ laboratory/industry for atleast one month with certificate from respective authority.
2. Techno-commercial case study of any four units from the following

Students will visit at least four units of the following to prepare a report for submission

1. Biofertilizer Unit
2. Mushroom cultivation unit
3. Green house unit
4. Floriculture unit
5. Plant nursery unit
6. Garden designing and maintenance unit
7. Fruit processing unit
8. Bio-pesticide unit
9. Biomass briquette unit
10. Biofuel units
11. Plant tissue culture industries
12. Farmhouse management
13. Pomoculture units
14. Organic farming
15. Fresh vegetables and flower supply unit



16. Herbal product industry
17. Forest department unit
18. Medicinal plant garden
19. Effluent treatment plant
20. Solid waste management unit





ESTD - 1928

A PROJECT ON

“Molecular and Phylogenetic Study of Some Selected Fungal Species of Order Polyporales”

A PROJECT SUBMITTED

To

SAVITRIBAI PHULE UNIVERSITY OF

FORTHE FULFILMENT OF

DEGREE IN BOTANTY

BY

MISS. WAGH GAYATRI BALKRUSHNA

UNDER THE GUIDANCE OF

Prof. Swapnil. D. Wagh

POST GRADUATE DEPARTMENT OF BOTANY

S.N.J.B.ASHRAM'S

KKHA ARTS, SMGL COMMERCE, SPHJSCIENCE (SENIOR) &
SPDS (JUNIOR) COLLEGE -CHANDWAD, DIST.- NASHIK, PIN-
423101

APRIL- MAY 2019





ESTD - 1928

CERTIFICATE

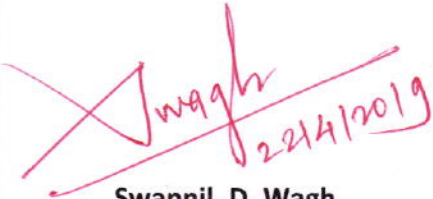
Seat no.:405381

This is to certify that the work incorporated in the project entitled "**Molecular and Phylogenetic Study of Some Selected Fungal Species of Order Polyporales**" being submitted by **Miss. Wagh Gayatri balkrushna wagh** for the award of **M.Sc Degree in Botany** from university of pune during the year **2018-2019** was carried out under the guidance & supervision of **Prof. Swapnil. D. Wagh.**

The matter embodied in this project has not been submitted part of or full to any other university for award of any degree.

Date: - / / 2018

Place:-Chandwad

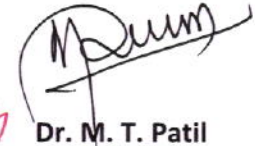

22/4/2019

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22/4

Examiner



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Department of Botany
HEAD

Department of Botany
SNJB's K.K.H.A.Arts, S.M.G.L. Commerce
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Chandwad-423 101 Dist-Nashik

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DECLARATION

I hereby declared that project entitled, "**Molecular and Phylogenetic Study of Some Selected Fungal Species of Order Polyporales**" submitted for the degree of M.Sc in botany has not been previously submitted by me for any degree or diploma of any other university.

Name:- WAGH GAYATRI BALKRUSHNA.

Date:-/ / 2018

Place:-Chandwad

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ACKNOWLEDGEMENT

I thank the Almighty God for the opportunity given to me to pursue the subject to bring out my project successfully.

Firstly, I would like to thank Prof.S .D.Wagh sir Department of Botany SNJB College, Chandwad.Also thankful to my co.guideDr.M.Y.Borde Department of Botany SavitribaiPhule Pune University. For providing all the facilities for our project work.

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I would also like to thank the teachers of SNJB College for supporting me for my project work.

It is an honor to thank my beloved parents and family members whose blessings and encouragement is the sole reason behind all my success. It is a pleasure to thank all my friends who were my backbone in making this project a great success.



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INTRODUCTION

Polyporales is the biggest order in Basidiomycetes. It includes around 3200 species Identified till now. Member of Polyporales play a vital role in the process of wood decay, resulting severe damage to the forest economy of our country. All the species of Polyporales are grow on bark and wood e.g. on wood of Neem, Pimpran, Saptparni, Goldmohar, Amba, Kachnar etc. They also cause white rot, where in Lignin is degraded and cellulose is partly degraded. These fungi decay wood by releasing cellulolytic and Lignolytic enzymes which specially digest complex organic components of wood like cellulose, hemicelluloses and lignin. It is therefore, very important to identifying the species of the group Polyporales that course a great damage to stem/timbers of valuable forest. (Mali, 2016)

Polyporales order was proposed by Rea, after Patouillard, for Basidiomycetes having macroscopic basidiocarps in which the hymenophore is flattened (Thelephoraceae), club-like (Clavariaceae), tooth-like (Hydnaceae) or has the hymenium lining tubes (Polyporaceae) or some times on lamellae, the poroid or lamellate hymenophores being tough and not fleshy as in the Agaricales (Ranadive, Jite, Ranade,&Vaidya, 2013). polyporales, a group of morphologically complex, terrestrial basidiomycetes. A phylogenetic classification for these wood rotting fungi is under progress, but the groups are possibly not monophyletic (Zjawiony, 2004). Non-gilled fungi (previously Aphyllorphorales) and heterobasidiomycetes are important groups of Basidiomycota comprising various forms of macroscopic fungi, of which many are saprobic wood decayers and thus frequently grow on logs, stumps, or other dead wood In addition, some grow on living trees and cause decay of non-functional heartwood, whereas others invade conducting plant tissue as parasites or are mycorrhizal with the roots of plants. (Ura, V, P, &Eviatar, 2010). The species of *Polyporales* occur on 500 types of woody trees species. Polyporales, an order of the Basidiomycetes are generally characterized by non-septatebasidia, persistent gymnocarpous and non-putrescent fruit bodies which normally are not lamellate. Amongst wood rotting mycobiota, Polyporales comprise a chief group that attack standing trees and fallen branches..(S, 2015).Polyporales order comprising 3200 species like *Earliella scabrosa*, *Ganoderma lucidum*, *Hexagoniaglabra*, *Inonotusbaumii*, *Peniophor acinerea*, *Phellinus cesatii*, *Schizoporaro seotingens*, *Microporus xanthopusetc*. The secondary metabolites of polypores exhibit a wide range of biological activities such as antimicrobial, antiviral, antifungal, anticancer,



cardiovascular, antiinflammatory, antioxidant, immune stimulating, nematocidal, and other activities, more than 75% of screened polypores showed strong antimicrobial activity. These activities are associated not only with small molecule secondary metabolites but also with high molecular weight cell wall polysaccharide. (Zjawiony, 2004). Savitribai Phule Pune University Spread over a 411 acres (1.66 km²) Savitribai Phule Pune University is a home to Pune's rarest flora. Pune University campus has rich vegetation like *Dalbergia melanoxylon*, this species of trees are lush green during the monsoons the other species present in the campus are *Bursera penicillata*, *Cycas zeylanica*, *Stercucu liaguttata*, *Olea uropaea* (European Olive), *Ficus benghalensis* etc. So fungal diversity in the campus is also tremendous.



OBJECTIVE

- 1) To study molecular relationship of some selected fungal species.
- 2) To understand their phylogeny.



RIVEW OF LITERATURE

National work on Polyporales:

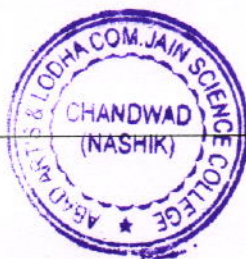
Studies on Polyporales were started along with the initiate of studies on Indian fungi. The first Indian record of a member of the Aphylophorales can be traced to Koltzsch(1832) in his paper on Indian Polyporaceae. Later Berkeley (1839) explained a some Indian polypores which were collected by W.J.Hooker. During the first quarter of the 20th century, Massee (1901, 1906, 1908, and 1910) published several accounts of Indian fungi based on collections sent to Kew Herbarium by several workers, notably by Sir E.J. Butler (1905a, b, c, d, 1918). Several Indian Aphylophorales were also reported by Lloyd (1898– 1925) and Sydow et al. (1906, 1907, 1911, 1912, 1916). Theissen (1913a,b) reported many PoroidAphylophorales collected from the Bombay presidency by Blatter. S.R. Bose (1919, 1923, 1924, 1925, 1927) was the first Indian mycologist to provide a broad account of the Indian polypores which he collected from Bengal and its surroundings. Sundaramani&Madurajan (1925) reported several members of Polyporaceae from Madras, and by 1925 there were more than 300 reports on the Aphylophorales (Fig. 2). Butler & Bisby (1931) made an assemblage of the Indian fungi in their classic work "The Fungi of India". This significant work encouraged the study of Indian fungi including Aphylophorales. Our knowledge about the Indian Aphylophorales was increased by the contributions of Bagchee&Bakshi (1950) Bagchee et al. (1954), Bakshi (1958, 1971), Bakshi et al. (1963), Puri(1956), Ramakrishan (1959), Rehill&Bashi (1965), Welden (1965), Reeves et al. (1967), Thind (1973, 1975), Sathe&Rahalker (1977), Rattan (1977), Thind&Dhanda (1978a,b), Anjali Roy (1979, 1981a,b, 1982, 1983, 1984, 1987), Harsh (1982), Natarajan& Raman (1980), Natarajan&Kolandavelu (1985), Vaidya (1987) Vaidya&Bhor (1990) Vaidya et al. (1991), Vaidya&Rabba (1993a,b), Rabba (1994), Sharma (1995) and Nanda (1996). (RanadiveKiran, 2013). Leelavathy& Ganesh (2000) conducted an extensive study on the polypores of Kerala and reported 73 species belonging to 26 genera. Florence & Yesodharan (2000) conducted a survey on macro fungi occurring in the Peechi-Vazhani Wildlife Sanctuary and reported 57 species belonging to 37 genera; out of this 35 species of polypores belonging to 24 genera were recorded. More recently Mohanan (2011) identified



and described a total of 89 species of polypores belonging to 32 genera from different forest ecosystems of Kerala.

International work on Polyporales :

The wood-rotting non-gilled agaricomycetes must have drawn the interest of man as the need for fire-wood arose, but it was Micheli (1729), who introduced the generic name *Polyporusto* include 14 species with centrally stalked fruit-bodies having pores on the bottom of the pileus. Hill (1751) introduced the generic name *Stereum*. He described three species of *Stereum* and made a record of 14 others. The first species is supposed to be composed of two species of *Stereum* viz *S. Hirsutum* (Wild. ex. Fr.) SF Gray and *S. purpureum* (Pers. ex Fr.) Fr. The genus was concealed by Linnaeus (1753) who combined it into *Boletus* . Linnaeus (1764) documented ten genera of fungi i.e. *Agaricus*, *Boletus*, *Hydnum*, *Phallus*, *Clathrus*, *Halvella*, *Peziza*, *Clavaria*, *Lycoperdon* and *Mucor* under the group, out of which seven are now regarded up 'Cryptogamia Fungi', which were mainly composite. The genus *Boletus* contained 14 species, out of which 7 are now regarded as members of poroid Agaricomycetes. The whole list of 93 species of fungi integrated only 9 polyporoid species *Agaricus (Daedalea) quercinus*, *A. (Lenzites) betulinus*, *Boletus suberosus (Polyporu sbetulinus)*, *B. (Fomes) fomentarius*, *B. (Fomes) igniarius*, *B. (Coriolus) versicolor*, *B. (Trametes) suaveolens*, *B. (Pycnoporus) sanguine* and *B. (Coltricia) perennis*. He did not separate fungi into orders or families but in 1780 he grouped the genera *Agaricus*, *Boletus*, *Hydnum*, and *Phallus* in a section "Pileati" and the remaining six in "Pileo destitute". Linnaeus (1780) characterized *Agaricus* as "subtuslamellosus", *Boletus* as 'subtusporosus', as *Hydnum* as subtusechinatus which laid foundation of the families. It may be mentioned here that family Polyporaceae had its inception here. Schaeffer (1762–1774) illustrated about 330 species of fungi in his traditional work and his ideas of classification are summarized in an index occupying seven (unnumbered) pages of fourth volume. He recognized only those genera already used by Linnaeus and each genus consisting a separate tribe. All poroid species of Polyporaceae were included in the tribe 'Boleti'. Willdenow proposed the generic name *Thelephora* (Thaelaephora). Persoon(1801), in his ' *Synopsis Methodica fungorum*' divided fungi into classes "Angiocarpi" and "Gymnocarpi". The latter was divided into three orders i.e. Lytothecii, Hymenothecii, and Naematothecii. The order Hymenothecii was divided into Agaricoidei, Boletoidi. Hydnoidei, Gymnodermata,



➤ PCR sequencing and identification

DNA amplification was carried out using ITS primer [ITS1 (forward primer) and ITS2 (reverse primer)].

PCR was performed with following cycles

- 1) 95° C for 4 min
- 2) 95° C for 1 min
- 3) 58° C for 1 min
- 4) 72° C for 1 min Repeat steps 2-4, 35 times
- 5) 72° C for 5 min
- 6) 4° C Forever

The PCR reaction having 25 µl total volume

. PCR COMPONENTS	Volume in (ul)
PCR Master-Mix	12.5
Nuclease Free Water	7.5
Forward Primer	1
Reverse Primer	1
Template DNA	3

➤ PCR analysis

After PCR reaction completion, the amplified PCR products are analyzed by agarose gel electrophoresis using 1.3% agarose. A molecular weight ladder is loaded into the first lane of the gel. 2ul of loading dye (containing GelRed as a dye) is mixed with 5ul of each PCR product and are loaded in separate wells on the gel. DNA fragment were run for 55 minutes cycle with an applied voltage 80V and current 91mA. The gel tray may be removed and placed directly under UV light to observe the bands.s



RESULT & DISCUSSION



1) Taxonomic Classification:

Kingdom-Fungi

Basidiomycota

Class-Agaricomycetes

Order-Polyporales

Family-Polyporaceae

Genus-Lenzites





2) Taxonomic Classification

Kingdom-Fungi

Division-Basidiomycota

Class-Agaricomycocetes

Order-Polyporales

Family-Polyporaceae

Genus-*Earliella*

Species-*scabrosa*





3)Taxonomic Classification

Kingdom-fungi

Division-Basidiomycota

Class-Agaricomycetes

Order-Polyporales

Family-ganodermataceae

Genus-*Ganoderma*

Species-*cornosum*





4) Taxonomic Classification

Kingdom-fungi

Division-Basidiomycota

Class-Agaricomycetes

Order-Polyporales

Family-ganodermataceae

Genus-*Ganoderma*

Species-*lucidum*





5) Taxonomic Classification

Kingdom-Fungi

Division-Basidiomycota

Class-Agaricomycetes

Order-Polyporales

Family-Polyporaceae

Genus-*Microporus*

Species-*vernicipes*



➤ Sequences Obtained By Sanger:

Earliella scabrosa

GCTACGTGCGTGACGGGCGTAGCTGGCTTCCGAGGCATGTGCACGCCCTGCTCATCC
ACTCTTACCCCTGTGCACTTACT
GTAGGCTTCAGGCGCGCTGGTTATTCAACGCCGTGACGATACTGGGTCTACGTTTTA
CTACCAACTACAAAGTATCAGAA
TGTGTATTGCGATGTAACGCATCTATATACTTTTTCAGCACGGATCTCTTGGCTCTC
GATCGATGAAGACGCAGCGCAG
TGGATACGTAATGTGAATTGCAGAATCAGTGATCATCGGTCTTTGACGCACTTGCGC
CGTTGTATCCGAGAGATGCTGGT
GAGTGCTGAATCTAGCTACAATCTTGC GGTTGAGCTGGCTGAGTTTGTGCTCCGCAG
CGCTCTCTAATCTACTGATCTGG
ACGCCTGTTGTATACTCGCCACTGAGGTGTGCTCTACGCCGTCAA AACTCTTACCTA
CCACAGAGATCCGTGCTACTTAT
GGAGAACGTTGAGGTAGGCTCAGTGCCCGCTCCTTCCGGGTTGGGTGCGGTAGTAG
ATGTGTTTTGGACAGTGAGGGTT

Ganoderma cornosum

GGGGCTTCGAGCTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTG
CTCAATCCACTCTACACCTGTGCACTTACTGTG
GGTGACGGATCGCAAAGCGGGCTCTTGTCCGTTATAAGCGCATCTGTGGCCTGCGTT
TACCACAAACTCTTTGAAAGTACTAGAATGTA
ATATTGGGATATAATAGATCTATATACTTTTTCAGCAACGGATCTCTTGGCTCTCG
CATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCC
TTGGTATTCCGAGGAGTATGCCTGTTTGTGAGTGTCATGAAATCTTCAACTTGCAACCTC
TTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTG



Ganoderma lucidum

GGCCTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTG
T
GGGCTTCAGATCGTAAAACGGGTCCCTTTACCGGGCTTGCGGAGCGTGTCTGTGCCG
CGTTTATCACAACTCTATAAAGTATCAGAATGTGTATTGCGATGTAACGCATCTAT
ATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAG
CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTT
GCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAAGTGTGCATGAAATCTTCAACCG
CAAGCTTTTGTGGTTTGTAGGCTTGAACCTTGGAGGCTTGTGCGCCGTTGTTGGTCGG
CTCCTCTTAAATGCATTAGCTTGGTTCCTTGC GGATCGGCTCTCAGTGTGATAATGTC
TACGCTGCGACCGTGAAGCGTCTGGCGAGCTTCTAACCGTCTCA

Microporus vernicipes

GGGGCTCCGGAACCTTTGATGGGTTGTAGCTGGCCTTCCGAGGGCATGTGCACGCC
TGCTCAATCCACTCTACACCTGT
GCACTTACTGTAGGTTTCTCGGTCGCGTTGGGTCTCTTCACTGGGGCTCGACAAAGC
CGAGGGGCTTATGTCTTACTACA
AACTATAAAGTAACTGAATGTATACCGCGTCTAACGCATCTATATACAACTTTCAGC
AACGGATCTCTTGGCTCTCGCAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACCTTG
CGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAAGTGTGCATGAAATTCTCAACCTA
CAAGCCTTTTTCGGAGGTCCTTGT
ACGGCTTGGACTTGGAGGGTCATTGTGCGGCAGTGATGTGCGGCTCCTCTTAAACGCAT
TAGCTAGTTCTCGCGGAACGGCT
TTCGGTGTGATAATTGTCTACGCCGTGGTCGTGCCGGGTACATGGACAAGCTTCTAA
ACCGTCACTCCTTGTGAGAGACACATATCTTGACATCTGACCTCAAATCAGGTAGGA
CTACCCGCTGAACTTACAGCATATCAATAGCCGGGAGGA

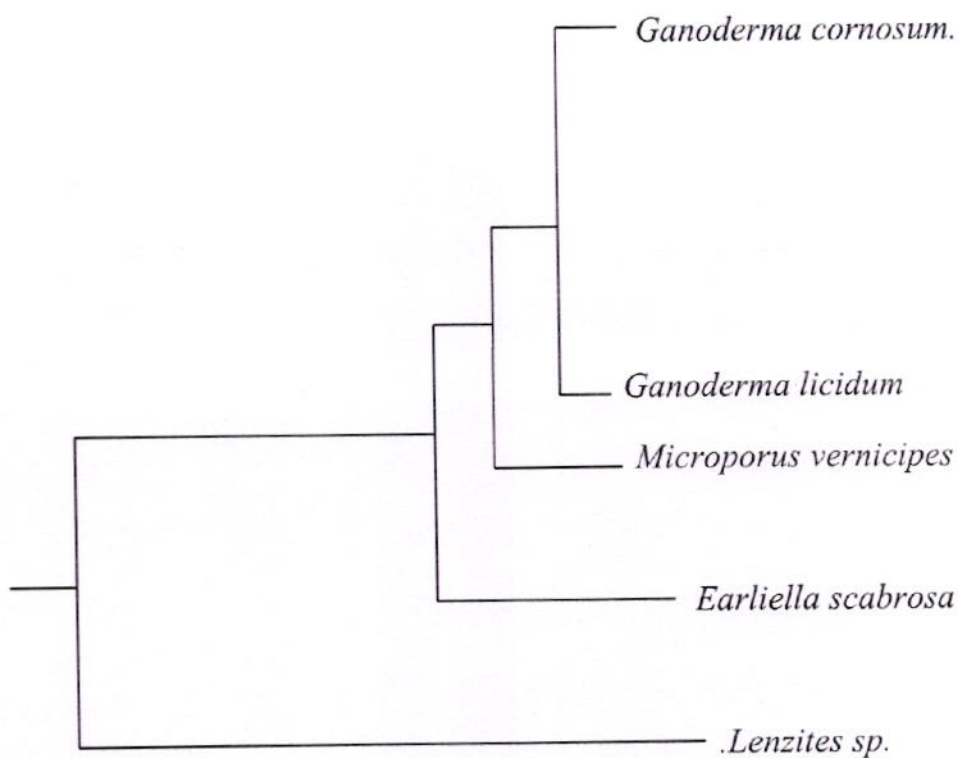


Lenzites sp.

TTGGCTTCGAGTTCTGCTGGGTTGTAGCTGGCCTTCGAGGATGTGCACACCCTGCCCT
TCCAGAAAGA
ATGTGGGTTTGGTTCGGCTTGGATGGTTTTGACGGTTACAGAACCGGCTACAAAATA
TCTTATATGTC
CCAACTTCCACATAAGAGGCTGCTCGAATACTTTTGAAAAATGCTTCCAGACGCATC
AAGTCCCCCTTC
CACCAGGATCCACGCTAATTCCTGTCCAGTACTTCTATTATACCATAATAACAAGTA
TTCAATCCGACTTA

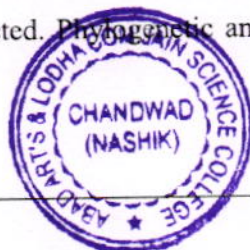


➤ Phylogenetic Tree



Five fungal specimens were collected from Savitribai Phule Pune University Campus belonging to order Polyporales. It consists of 3 families - Ganodermaceae, Polyporaceae, and Hymenochaetaceae. *Ganoderma* belongs to Ganodermaceae family, to Hymenochaetaceae and *Microporus*, *Favolus*, *Lenzites*, *Earliella*, *Hexagonia*, *Podoscypha*, *Trametes*, *Royoporus* belonging to family Polyporaceae.

Five fungal specimens were identified by morphological characters, of which five specimens were confirmed by molecular technique. The sequences obtained after outsourcing of PCR products were blasted in NCBI. The identified species sequences were taken in FASTA format and the phylogenetic tree was constructed. Phylogenetic analysis based on ITS sequence. Distance



and clustering with the neighbor -joining method by using the software MEGA 7. Bootstrap values based on 1000 replications are listed at percentages at the branching points. Phylogenetic analysis showed that *Favolus roseus* and *Microporus vernicipes* are evolved from common ancestor while *Microporus vernicipes* and *Lenzites sp.* were evolutionarily different



CONCLUSION

Present study intensely discussed the relationship and affinities between the major genera of Polyporales. This work has also been given notes on phylogenetic relationship between *Ganoderma cornosum*, *Ganoderma ligidum*, *Microporus vernicipes*, *Earliella scabrosa*, *Lenzites sp.* Phylogenetic analyses resulted in successful construction of a tree shows phylogeny. Morphological and molecular identification of collected members of Polyporales was done and established a phylogenetic, generic and species from DNA sequence data.



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