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# CHARACTERIZATIONS OF TREE-DECAY FUNGI BY MOLECULAR AND MORPHOLOGICAL INVESTIGATIONSIN ANIRANIAN ALAMDARDEH FOREST

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## ABSTRACT

Forest trees are considered important in ameliorating climate change through removing carbon dioxide from the atmosphere, stabilizing water catchments and for timber production. Wood decay fungi are among the most important biotic factors in ecosystems, infecting valuable landscaping trees causing an economic loss or the preeminent recyclers of the wood. In a survey of forest trees in the Alamdardeh forest, northern Iran, fungal fruit bodies were collected and isolations made. Based on a combination of macro-morphological characteristics and molecular analyses, using the sequence data of ITS-rDNA, isolates were identified to the species level. A total of 22 species in nine families and 15 genera were identified. Most isolates were the white-rot fungi. Additionally, the brown-rot fungus *Laetiporus sulphureus* and the soft-rot species *Xylaria longipes* were identified.

**Keywords:** Brown-rot fungi, fungal fruit, Alamdardeh forest trees, molecular identification, rDNA-ITS sequencing, tree rot fungi, white-rot fungi.

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## INTRODUCTION

Forest trees provide numerous ecological functions, such as oxygen production, carbon dioxide sequestration, prevention of soil erosion, water catchment management, protection of biodiversity, and multiple benefits for humans. Forests are managed for traditional forest products such as timber for construction or firewood, and fiber for paper manufacture (Young 1982). The northern forests of Iran are temperate and include species of oriental beech (*Fagus orientalis*), chestnut-leaved oak (*Quercus castaneifolia*), common hornbeam (*Carpinus betulus*), caucasian alder (*Alnus subcordata*) and velvet maple (*Acer velutinum*). In forest ecosystems, fungi play a fundamental role in recycling nutrients, thereby providing a vital function in natural forest ecosystems (Aghajani *et al.* 2017). However, forests can also be damaged by fungi, bacteria, insects, and parasitic plants. Amongst these damaging agents, wood inhabiting and/or decay fungi are important, particularly the white and brown-rot species, although their functioning may balance forest ecosystems.

Aghajani *et al.* (2013) presented a comprehensive report of the wood-inhabiting fungi in northern Iran, identified by morphological features. Alamdardeh forest, part of the Mazandaran forests of northern Iran, has a high biodiversity of tree species, dominated by oriental beech (*Fagusorientalis*), chestnut-leaved oak (*Q. castaneifolia*), common hornbeam (*C. betulus*), caucasian alder (*A. subcordata*) and velvet maple (*A.velutinum*).

Classical mycological identification methods including those based on morphological features such as type of decay, fruit body characterization, spore and mycelium morphology (e.g. Nobles 1965, Stalpers 1978, Lombard and Chamuris 1990) are unsuitable for the definitive identification of species in many genera, particularly *Armillaria*, *Ganoderma* or *Pleurotus*. In the last approximately 25 years, molecular analytical techniques have been developed for rapid and more stringent identification of wood inhabiting fungi, with methods such as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis; Schmidt and Kebernik 1989, Vigrow *et al.* 1991), RAPD (random amplification of polymorphic DNA; Schmidt and Moreth 1998a), RFLP (restriction fragment length polymorphism; Schmidt and Moreth 1998b), species-specific priming PCR (Schmidt and Moreth 1999, Schmidt and Moreth 2000), sequencing of the rDNA-ITS region (e.g. White *et al.* 1990, Schmidt and Moreth 2002, Kauserud *et al*. 2004), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Schmidt and Kallow 2005, Pristaš *et al.* 2017) and their degradation behaviors (Bari *et al.* 2021). For example, different researchers (Luley 2005, Terho *et al.* 2007, Schmidt *et al.* 2012) identified several fungi associated with rot in urban trees in the United State, Finland, and Germany, respectively, using ITS-sequencing.

Alamdardeh, within the Kiasar forest area, is an example of an old-growth forest in northern of Iran. This area has a high biodiversity of tree species. The dominant species are oak and hornbeam, with a mixture of *F. orientalis* and *A subcordata*. The average age and diameter of oak and hornbeam trees are 150 and 100 years old, and 75 cm to 250 cm, respectively. In the forests of Iran, Bari (2014) previously collected *Trametes versicolor* and *Pleurotus ostreatus*, causing white rot on beech (*F. orientalis*)in this forest, with identification by ITS-rDNA sequencing. The properties of beech, oak, and spruce wood decayed by both white-rot fungi were determined (Bari *et al*. 2015a, Bari *et al*. 2015b, Bari *et al*. 2015c, Bari *et al*. 2015d, Bari *et al*. 2016, Bari *et al*. 2017, Bari *et al*. 2018, Bari *et al*. 2020, Aghajani *et al.* 2018). In previous work, several *Xylaria* species were also identified using ITS-rDNA from trees occurring in the Gilan forest (Hashemi *et al.* 2015). The aim of the work described in this paper was to increase knowledge of the fungi causing decay of forest trees in Alamdardeh forest, using isolates collected from both standing and fallen trees, through identification using ITS-rDNA sequencing. Accurate identification provides valuable information about the impact of decay on the trees, precise estimation of the distribution of fungal saprophytes and pathogens causing wood decay on standing and fallen trees, mode of action and importance to risk analysis.

## MATERIAL AND METHODS

### Study site

The broad-leaved deciduous forest forming a 20 km to 70 km wide and 800 km long belt parallel to the southern coast of the Caspian Sea was examined. Alamdardeh forest is located at 39°70‘ to 39°74‘N, 40°24‘ to 40°40‘E. These forests cover the northern slopes of the Alborz Mountains of northern Iran, extending from the Caspian lowlands to an elevation of 2800 m and covering an area of ca. 1,9 million ha (Marvie Mohadjer

2011).

**Fungal isolation**

Fungal sampling was carried out between 2012 - 2015, as described by Aghajani (2012). Samples of colonized wood, along with carpophores from trees including oak (*Quercus castaneifolia* C.A. Mey.), hornbeam (*Carpinus betulus* L.), maple (*Acer velutinum* Boiss), and beech (*Fagus orientalis* Lipsky.) were collected from standing and fallen trees, and stored at 4°C for transfer to the laboratory.

**Photography**

Fruit bodiescollected from host trees were photographed with a high definition Canon IXY 50S camera (Japan) and images transferred to Image J software (ImageJ 2020) for analysis.

**DNA extraction, polymerase chain reaction and sequencing**

Fruit bodies were initially identified by macro- and microscopic analysis (Ryvarden and Gilbertson 1993, Ryvarden and Gilbertson 1994). Molecular identification was performed following the methods of Schmidt *et al.* (2012), Bari *et al.* (2017), Aghajani *et al.* (2018): Approximately 20 mg tissue was taken from the interior of aseptically opened fruit bodies with flamed forceps. DNA was extracted by grinding the tissue using the DNeasy Plant Mini Kit (Denazist, Mashhad, Iran). DNA concentration was measured by UV spectrophotometry and proportional dilutions made. Polymerase chain reaction (PCR) was used to amplify the ITS-rDNA region using the ITS4 and ITS5 primer sets as forward and reverse primers, respectively (White *et al.* 1990). All PCR reactions were prepared in a total volume of 25 µl, comprising 50 ng genomic DNA mixed with 1× CinnaGen PCR Master-mix (CinnaGen, Tehran, Iran) and 0,2 µM of each primer. The PCR protocol was: initial denaturing of 4 min at 98 °C, 35 cycles of 30 sec at 94 °C for denaturing, 30 sec at 58 °C for annealing, 1 min at 72 °C for extension, and a final extension of 7 min at 72 °C. Aliquots of PCR products were examined on 2 % agarose gels stained with GelStar Nucleic Acid Gel Stain (Lonza Rockland, Inc, USA) and examined under UV light. PCR products for sequencing were sent to Takapouzist Co. (Bioneer, Korea). Species were identified by sequence comparison with accessions in the NCBI databases using the BLAST program.

**Phylogenetic analysis**

Forward and reverse ABI raw trace files were used to create consensus sequences using the Staden package program, version 2.0.0b9-src.tar.gz (Staden 1996). Consensus sequences were used as queries to blast (Mega BLAST from NCBI) the GenBank nucleotide database. Sequences with the highest similarity together with reference strains were downloaded from GenBank and aligned using MUSCLE software (Edgar 2004) implemented in MEGA6 (Tamura *et al.* 2013). The best evolutionary model for the alignments was calculated using MrModelTest software, v. 2.3 (Nylander 2004). Bayesian inference (BIs) was used to build phylogenetic trees using MrBayes v. 3.2.1 (Ronquist and Huelsenbeck 2003). Two separate BIs were run for three datasets, the Ascomycota (Xylariales) and the Basidiomycota (Agaricales and Polyporales). For each of the two BIs, the heating parameter was set at 0,15 and four Markov Chain Monte Carlo (MCMC) chains were run, starting from random trees for 1 million generations, with trees sampled every 1,000 generations. The first 25 % of trees were discarded as burn-in; consensus tree and posterior probabilities (PP) were determined from the remaining trees. Phylogenetic trees were inspected and printed using Fig Tree ver. 1.3.2 (Rambaut 2009). Trees were rooted using *Nectriacinnabarina* CBS 279,48 and *Podoserpula pusio* AFTOL-ID 1522 for ascomycetous and basidiomycetous fungal taxa, respectively. Sequences derived from this work were deposited in the NCBI GenBank nucleotide database (Table 1).

**Table 1:** Fungal strains used for phylogenetic analysis with their GenBank accessions.

| Order | Family | Species | Collection ID. | ITS GenBank Accession NO. | Source[a] |
|---|---|---|---|---|---|
| Xylariales | Xylariaceae | *X. hypoxylon* | CBS 122620 | KY204024 | CBS |
| | | *X. hypoxylon* | RP432 | KX096696 | DSMZ |
| | | *X. hypoxylon* | STF132 | MG835884 | STF (this study) |
| | | *X. longipes* | CBS 147,73 | AY909017 | CBS |
| | | *X. longipes* | CBS 148,73 | AF163038 | CBS |
| | | *X. longipes* | INBio:612C | KU204608 | INBio |
| | | *X. longipes* | STF133 | MG835885 | STF (this study) |
| | | *X. polymorpha* | STF134 | MG835886 | STF (this study) |
| | | *X. polymorpha* | NBio:1143C | KU204440 | INBio |
| | | *X. polymorpha* | IFO 9780 | AF163041 | IFO |
| | | *X. polymorpha* | CBS 590,72 | MH860591 | CBS |
| | | *K. deusta* | CBS 163,93 | KC477237 | CBS |
| | | *K. deusta* | STF118 | MG835868 | STF (this study) |
| Agaricales | Physalacriaceae | *A. mellea* | AFTOL-ID 448 | AY789081 | AFTOL |
| | | *A. mellea* | STF110 | MG835859 | STF (this study) |
| | Pleurotaceae | *P.pulmonarius* | 4203 | AY450349 | TENN |
| | | *P. pulmonarius* | ZBS2012 | KF932728 | ZBS MSU |
| | | *P. pulmonarius* | STF123 | MG835875 | STF (this study) |
| | | *P. ostreatus* | 6689 | AY450345 | TENN |
| | | *P. ostreatus* | 38d | JQ837475 | ZBS MSU |
| | | *P. ostreatus* | CBS 291,47 | EU424309 | CBS |
| | | *P. ostreatus* | STF122 | MG835874 | STF (this study) |
| | Strophariaceae | *P. aurivella* | TENN61741 | MH855317 | TENN |
| | | *P .aurivella* | CBS 118,18 | MH854669 | CBS |
| | | *P.aurivella* | STF121 | MG835872 | STF (this study) |
| | Schizophyllaceae | *S.commune* | SCAU126 | AY636062 | SCAU |
| | | *S. commune* | BCC22128 | FJ372688 | BCC |
| | | *S. commune* | STF127 | MG835879 | STF (this study) |
| Polyporales | Polyporaceae | *G.adspersum* | CBS 351,74 | EU162053 | CBS |
| | | *G. adspersum* | HSBU-200894 | MG279154 | BJFC |
| | | *G. adspersum* | GAD3 | JN222418 | CMI-Unibo |
| | | *G. adspersum* | STF113 | MG835862 | STF (this study) |
| | | *G. applanatum* | K(M)120829 | AY884179 | BJFC |
| | | *G. applanatum* | SFC20141001-25 | KY364256 | SFC |
| | | *G. applanatum* | STF114 | MG835863 | STF (this study) |
| | | *G. lucidum* | STF116 | MG835865 | STF (this study) |
| | | *G. lucidum* | G1T099 | AM269773 | Di.Va.P.R.A. |
| | | *G. lucidum* | HMAS86597 | AY884176 | BJFC |
| | | *C. trogii* | SYBC-LZ | HQ000043 | SYBC |
| | | *C. trogii* | TEM H2 | HM989941 | TEM |
| | | *C. trogii* | STF111 | MG835860 | STF (this study) |
| | | *F. fomentarius* | FF-TdQ-br | AY849305 | CRA-PAV |
| | | *F. fomentarius* | 255FT_SSI | JX126890 | NCSLG |
| | | *F. fomentarius* | STF112 | MG835861 | STF (this study) |
| | | *C. squamosus* | STF126 | MG835878 | STF (this study) |
| | | *C. squamosus* | Wang555 | KU189779 | BJFC |
| | | *C. squamosus* | Cui10595 | KU189778 | BJFC |
| | | *L. betulinus* | CBS 695,94 | JN645081 | CBS |
| | | *L. betulinus* | ASIS22871 | KF692081 | NAAS |
| | | *L. betulinus* | STF128 | MG835880 | STF (this study) |
| | | *T. gibbosa* | STF129 | MG835881 | STF (this study) |
| | | *T. gibbosa* | CFMR:DLL2011-045 | KJ140568 | CFMR |
| | | *T. gibbosa* | CBS 284,30 | MH855141 | CBS |
| | | *T. hirsuta* | BRFM<FRA>:994 | JN645100 | BRFM |
| | | *T. hirsuta* | CBS 282.73 | MH860685 | CBS |
| | | *T. hirsuta* | STF130 | MG835882 | STF (this study) |
| | | *T. versicolor* | BRFM<FRA>:1219 | JN645058 | BRFM |
| | | *T. versicolor* | CBS 122155 | DQ674379 | CBS |
| | | *T. versicolor* | STF131 | MG835883 | STF (this study) |
| | Irpicaceae | *I. lacteus* | STF117 | MG835867 | STF (this study) |
| | | *I. lacteus* | CBS 431,48 | AY569565 | CBS |
| | | *I. lacteus* | KUC8604 | JX290571 | KUC |
| | Meruliaceae | *P. radiata* | SFC20151020:13 | MF437006 | SFC |
| | | *P. radiata* | ATCC 64658 | FJ746663 | ATCC |
| | | *P. radiata* | STF120 | MG835870 | STF (this study) |
| | Laetiporaceae | *L. sulphureus* | KATRIN-3 | EU840607 | SUN |
| | | *L. sulphureus* | RVP4 | EU840599 | SUN |
| | | *L. sulphureus* | ERT-713 | EU402564 | CFMR |
| | | *L. sulphureus* | STF119 | MG835869 | STF (this study) |
| out-group | Nectriaceae | *Nectria cinnabarina* | CBS 279,48 | AF163025 | CBS |
| out-group | Amylocorticiaceae | *Podoserpula pusio* | AFTOL-ID 1522 | DQ494688 | AFTOL |

[a]AFTOL:Assembling the Fungal Tree of Life, USA; ATCC: American Type Culture Collection, Manassas, VA, USA; BCC: Bioresources Techonology Unit, National Center for Genetic Engineering and Biotechnology, Thailand; BJFC: Institute of Microbiology, Beijing Forestry University, China; BRFM: Banque de Ressources Fongiques de Marseille, France; CBS: Westerdijk Fungal Biodiversity Institute, Netherland; CFMR: Center for Forest Mycology Research herbarium, Wisconsin, USA; CMI-Unibo: Mycological Herbarium of the Mycological Center of the University of Bologna, Italy; CRA-PAV: Centro di Ricerca per la Frutticoltura di Roma, Italy; Di.Va.P.R.A: Department of Exploitation and Protection of the Agricultural and Forestry Resources, University of Torino, Grugliasco, Italy; DSMZ: DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IE²: Institute of Ecology and Evolution, University of Oregon,USA; IFO: Institute for Fermentation, Osaka, Japan; INBio: National Institute for Biodiversity and Conservation, Costa Rica; MSU: Department of Mycology and Algology, Lomonosov Moscow State University, Russia; KUC:Korea University Culture collection, South Korea; NAAS: National Academy of Agricultural Science, Microbiology, South Korea; NCSLG: Mycological Herbarium at North Carolina State University, USA; SCAU:South China Agricultural University, China; SFC: Seoul National University Fungus Collection, South Korea; STF (TVU): Sari Technical Faculty, Iran; SUN: College of Natural Science, Seoul National University, South Korea; SYBC: School of Biotechnology, Jiangnan University, China; TEM:Biology Department, Ege University, Izmir, Turkey; TENN: University of Tennessee Herbarium, USA; ZBS MSU: Zvenigorod Botanical Station, Moscow State University, Moscow Region, Russia.

## Results and discussion

### Identification of fungi

Fungi identified from trees in the Alamdardeh forest, northern Iran is presented in Table 2, Figure 1 and Figure 2. Twenty two species of decay fungi were identified on standing and fallen trees in the forest (Figure 1 and Figure 2). A total of 122 specimens with most of the specimens being collected most fungi identified were white-rot species, with two species causing brown-rot (*Laetiporus sulphureus*) or soft-rot (*Xylaria longipes*) (Table 2, Figure 1 and Figure 2).

**Table 2:** Fungal taxa recognized with their hosts, sampling date, rot type, and frequency.

| Fungus | Host/Occurrence | Sampling | Type of rot | Frequency |
|---|---|---|---|---|
| *Armillaria mellea* (Vahl) P. Kumm. | *Carpinus betulus*/standing | Nov-16 | WR | 5 |
| *Coriolopsis trogii* (Berk.) Domański | *Carpinus betulus*/standing | Jan-16 | WR | 7 |
| *Fomes fomentarius* (L.) J. Kickx f. | *Carpinus betulus*/standing | Nov-16 | WR | 12 |
| *Ganoderma adspersum* (Schulzer) Donk | *Quercus castaneifolia*/standing | Oct-17 | WR | 5 |
| *Ganoderma applanatum* (Pers.) Pat. | *Quercus castaneifolia*/standing | Oct-15 | WR | 3 |
| *Ganoderma lucidum* (Curtis: Fr.) P. Karsten | *Carpinus betulus*/standing | Jan-17 | WR | 7 |
| *Irpex lacteus* (Fr.) Fr. | *Quercus castaneifolia*/fallen | Jan-15 | WR | 3 |
| *Kretzschmaria deusta* (Hoffm.) P. D. M. Martin | *Carpinus betulus*/dead standing | Nov-14 | WR | 2 |
| *Laetiporus sulphureus* (Bull.) Murrill | *Quercus castaneifolia*/standing | Nov-14 | BR | 2 |
| *Phlebia radiata* Fr. | *Quercusc astaneifolia*/fallen | Jun-14 | WR | 1 |
| *Pholiota aurivella* (Batsch.) P. Kumm. | *Fagus orientalis*/fallen | Jun-17 | WR | 2 |
| *Pleurotus ostreatus* (Jacq.) P. Kumm. | *Fagus orientalis*/fallen | Sep-14 | WR | 4 |
| *Pleurotus pulmonarius* (Fr.) Quél. | *Quercus castaneifolia*/fallen | Jun-16 | WR | 1 |
| *Polyporus squamosus* (Huds.) Fr. | *Acer velutinum*/fallen | May-14 | WR | 3 |
| *Schizophyllum commune* Fr.: Fr. | *Acer velutinum*/fallen | Jul-17 | WR | 12 |
| *Lenzites betulinus* (Fr.) Fr. | *Quercus castaneifolia*/fallen | Jan-15 | WR | 5 |
| *Trametes gibbosa* (Pers.) Fr. | *Carpinus betulus*/fallen | Jan-17 | WR | 13 |
| *Trametes hirsuta* (Wulfen) Pilat | *Quercus castaneifolia*/fallen | Jan-15 | WR | 6 |
| *Trametesversicolor* (L.) Lloyd | *Carpinus betulus*/fallen | Jun-16 | WR | 5 |
| *Xylariahypoxylon* (L. and Hook.) Grev. | *Carpinus betulus*/fallen | Jun-16 | WR | 1 |
| *Xylaria longipes*Nitschke | *Carpinus betulus*/fallen | Jun-14 | SR | 2 |
| *Xylaria polymorpha* (Pers. and Mer.) Grev. | *Carpinus betulus*/fallen | Jan-15 | WR | 21 |

In a total of 122 specimens, most specimens were collected from *Carpinus betulus* followed by *Quercus castaneifolia, Fagus orientalis* and *Acer velutinum* (Table 2), whereas the corresponding sequence was *Xylaria polymorpha*, *Trametes gibbosa*, *Fomes fomentarius* and *Schizophyllum commune* for the frequency of obtained fungal taxa (Table 2).

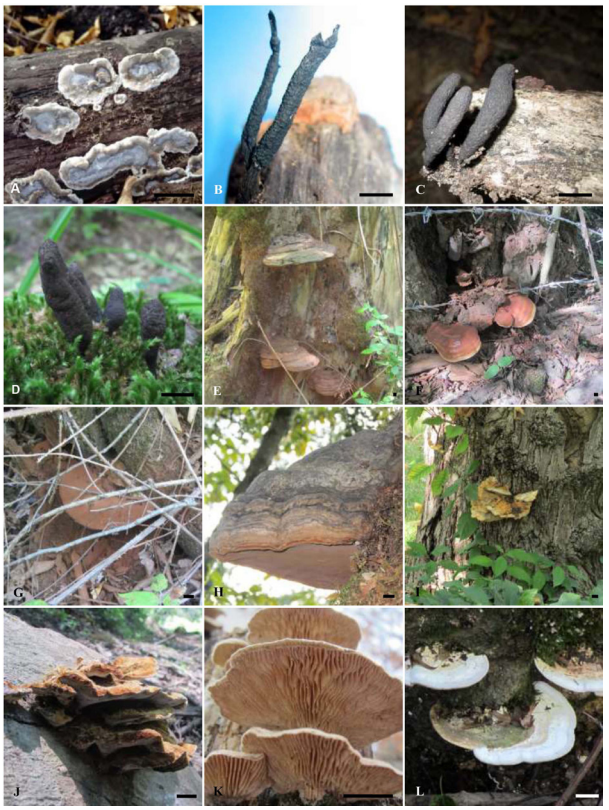

**Figure 1:** Fruit bodies of Ascomycota and Basidiomycota from trees in Alamdardeh forest, northern Iran. A: *Kretzschmaria deusta*, B: *Xylaria longipes*, C: *X. hypoxylon*, D: *X. polymorpha*. E: *Ganoderma adspersum*, F: *G. Lucidum*, G: *G. applanatum*, H: *Fomes fomentarius*, I: *Laetiporus sulphureus*, J: *Coriolopsis trogii*, K: *Lenzites betulinus*, L: *Trametes gibbosa*.
Scale bars: 2cm.

**Figure 2:** Fruit bodies of Basidiomycota from trees in Alamdardeh fores. A: *Trametes hirsuta*, B: *T. versicolor*, C: *Pleurotus.pulmonarius*, D: *P. ostreatus*. E: *Armillaria mellea*, F: *Schizophyllum commune*, G: *Pholiota aurivella*, H: *Irpex lacteus*, I: *Cerioporus squamosus*.
Scale bars: 2cm.

**Phylogenetic analysis**

The aligned ITS datasets for Xylariales (Ascomycota), Agaricales and Polyporales (Basidiomycota) contained 13, 15 and 41 in-group taxa with 557, 822 and 714 characteristics containing 172, 401 and 399 unique site patterns respectively. MrModeltest v. 2.3 found GTR+G+I to be the most fitting replacement models for both ITS datasets. The Bayesian analysis enabled the identification of four Ascomycota as *Kretzschmaria deusta, Xylaria longipes, X. hypoxylon*, and *X. Polymorpha* with the highest posterior probability (Figure 3, Figure 4 and Figure 5).

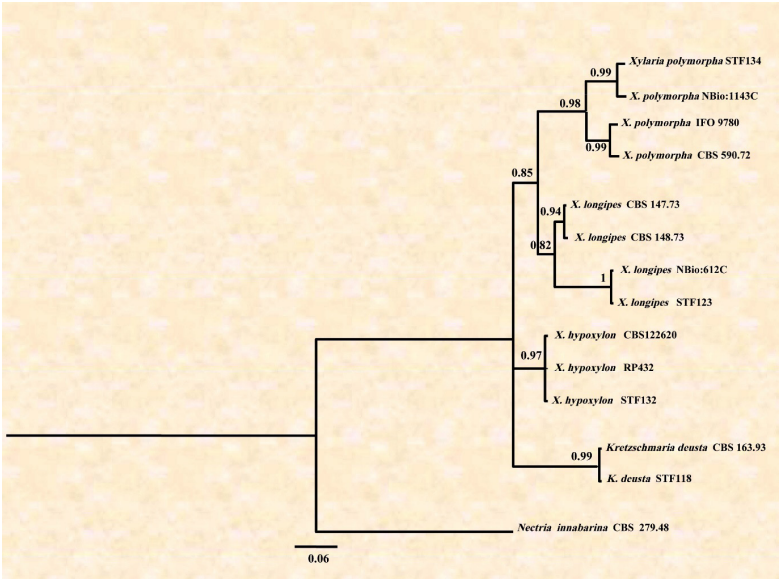


**Figure 3:** ITS phylogeny of different fungal taxa belonging to Xylariales (Ascomycota), including the sequences generated in this study marked as STF (TVU), using Bayesian inference based on the GTR+G+I model.The scale bar shows 0,06 expected changes per site. The tree was rooted to *Nectria cinnabarina* (CBS 279,48).

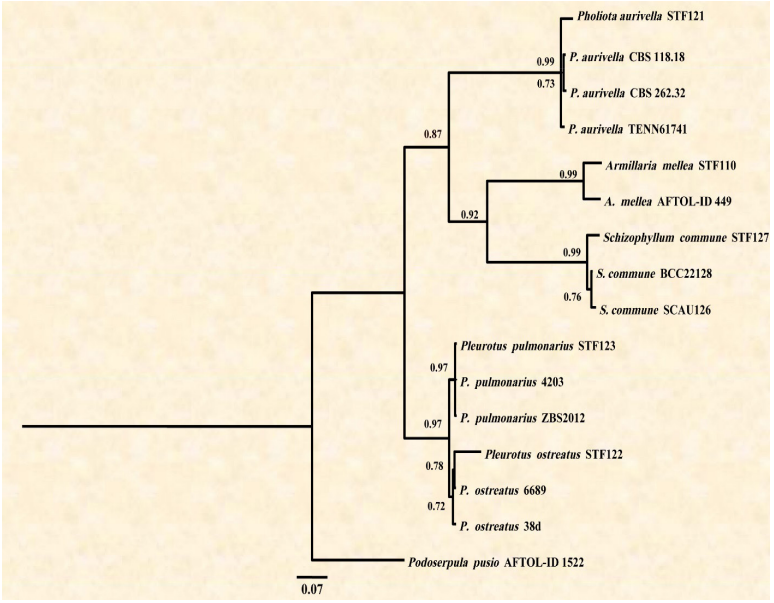


**Figure 4:** ITS phylogeny of different fungal taxa belongin to Agaricales (Basidiomycota), including the sequences generated in this study marked as STF (TVU), using Bayesian inference based on the GTR+G+I model. The scale bar shows 0,07 expected changes per site. The tree was rooted to *Podoserpula pusio* (AFTOL-ID 1522).

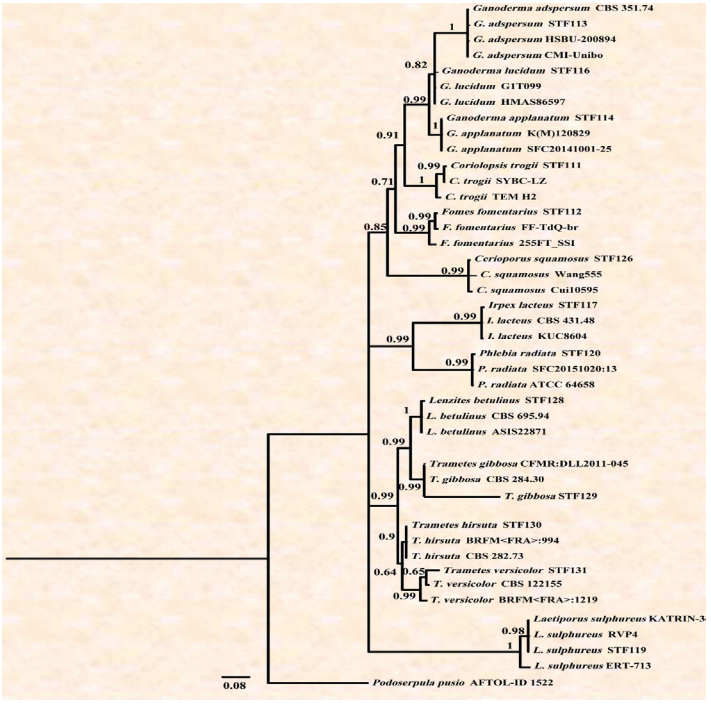


**Figure 5:** ITS phylogeny of different fungal taxa belongin to Polyporales (Basidiomycota), including the sequences generated in this study marked as STF (TVU), using Bayesian inference based on the GTR+G+I model.The scale bar shows 0,08 expected changes per site. The tree was rooted to *Podoserpula pusio* (AFTOL-ID 1522).

Moreover, the identity of eighteen Basidiomycetous fungal taxa belonging to the orders of Agaricales (*Armillaria mellea*, *Pleurotus ostreatus, P. pulmonarius*, *S. commune* and *Pholiota aurivella*) and Polyporales (*Coriolopsis trogii, F. fomentarius, Ganoderma adspersum, G. lucidum, G. applanatum, Irpex lacteus, L. sulphureus, Lenzites betulinus, Phlebia radiata, Cerioporus squamosus* (formerly known as *Polyporus squamosus*), *T. gibbosa, T. hirsuta* and *T. versicolor*) (Figure 1 and Figure 2) were identifiedusing Bayesian analysis inference. Overall, all fungal taxa examined in this study were placed in 22 distinct clades with the highest posterior probability (Figure 3, Figure 4 and Figure 5).

Using a combination of macro-morphological characteristics and molecular phylogeny, a total of 4 ascomycetousand 18 basidiomycetous fungal taxa were identified (Figure 1, Figure 2, Figure 4 and Figure 5). Bayesian inference of ITS-rDNA revealed the identity of the fungal taxa obtained with the highest posterior probability (Figure 3, Figure 4 and Figure 5). Phylogenetic analyses based on the sequence data of ITS-rDNA have been previously proved to be practical for the identification of fungi of highly variable morphology like *Xylaria* spp. and *Ganoderma* spp. (Cao *et al.* 2012).

Since the advent of DNA-based identification using PCR (Mullis and Faloona 1987), molecular techniques have been developed for efficient and reliable detection of fungi in plant tissues. Earlier molecular methods used for identification of wood-inhabiting fungi (Schmidt and Kebernik 1989, Schmidt and Moreth 1998a, Schmidt and Moreth 1998b), including SDS-PAGE, RAPD and RFLP, were less suitable for unknown fungal species because very different species can yield similar results by chance; these techniques should only be used if the species in question is already pre-identified by other methods or for revealing within-isolate polymorphism among the populations of fungal species. Species-specific PCR primers (Schmidt and Moreth 1999, Schmidt and Moreth 2000) can identify unknown species; however, development is time-consuming and the ITS sequences of species within some genera (e.g. *Armillaria*) are too similar for standard primers to separate. For example, ITS sequences of *Armillaria borealis*, *A. cepistipes*, *A. gallica*, and *A. ostoyae* showed considerable similarity, but differed from *A. mellea* (Potyralska *et al.* 2002). However, since 2000, sequencing of the ITS-rDNA region as a molecular barcode and subsequent species identification by sequence comparison with ITS depositions in DNA databases has commonly been used for detection of unknown fungi; the technique is rapid, gives confidence in the results, and numerous ITS sequences for identification, by comparison, are available in DNA databases.

In this study, most fungi identified (Table 1) were white-rot species, with two species causing brown-rot (*Laetiporus sulphureus*) or soft-rot (*Xylaria longipes*) (Table 1). White-rot fungi are common in hardwoods, whereas brown-rot species prefer softwoods (Schmidt 2006). White rot fungi degrade the lignin component of the wood in the first stages of the degradation, and then cellulose and hemicellulose while brown rot fungi only degrade the carbohydrates cellulose and hemicellulose (Pandey and Pitman 2003). Since Iranian forests are dominated by angiosperm trees, white-rot fungi are common. Several of the fungal species identified were also reported by Schmidt *et al.* (2012) from urban trees in northern Germany. These results correspond to Ryvarden and Gilbertson (1993) and Ryvarden and Gilbertson 1994, who suggested that many decay species are cosmopolitan.

It is known that changes in the quantity and also the quality of fallen and standing dead trees in managed and unmanaged forests result in variations in the fungi present (Marvie Mohadjer 2011). Moreover, dead and fallen trees in forest ecosystems provide habitats and substrates for fungal species and other organisms that live in and on the wood. For example, snags have major roles in the localised distribution of macro-fungi and they are known to be of great value in managed forests, and therefore, it is recommended that several standing dead trees are left during harvesting operations (Aghajani *et al.* 2016). Aghajani *et al.* (2013) studied wood-inhabiting fungi in Kheyroud forest (Mazandaran province), which has different climate conditions compared to the current work, and found high variations in fungal taxa such as *Armillariamellea*, *Stereum* sp., *Pluteus cervinus*, *Ganoderma applanatum*, *Trichaptum* sp., *Fomes fomentarius*, *Pluteus* sp., and *Schizophyllum commune* on oak, and *A. mellea*, *Hypholoma fasciculare*, *Crepidotus* sp., *Pluteus* sp., *Coprinus* sp., *G. applanatum*on hornbeam, representing first reports for Iran (Aghajani *et al.* 2013, Aghajani *et al.* 2014).

Generally, many of the above fungi were found in the current work, indicating the selective effect of the host tree on the presence and distribution of fungi. Another factor that potentially led to random variation in the present study was that most dead wood units were surveyed only once, thus a number of species may have remained undetected (Halme and Kotiaho 2012, Abrego *et al.* 2016). It is likely, therefore, that there are more rare species (with few occurrences) than suggested in the dataset presented here. Nevertheless, as has been shown by both molecular (Kubartova *et al.* 2012) and fruit body based surveys (Abrego *et al.* 2016), a high proportion of rare species is an inherent characteristic of wood-inhabiting fungal communities. For some species, the geographical regions examined captured most of the variation observed; meaning that after accounting for variables related to climate, forest connectivity and resource quality, the presence of these fungi was mainly confined to particular geographical areas. However, the use of developed techniques of DNA-based identification including multi-gene and metagenomic identification of environmental DNA is sometimes inevitably necessary to reveal rare and invasive species within a habitat (Stewart *et al.* 2018). Because many wood-associated fungi are morphological similar to each other or cause similar symptoms on their hosts and also obligate forest pathogens are unable to grow on synthetic cultures (Stewart *et al.* 2018).

## CONCLUSIONS

The accurate knowledge of fungal species associated with wood decay such as those identified in this study could be further helpful to adopt proper management of the forests. A total of 22 fungal taxa associated with wood decay in standing and fallen trees in the Alamdardeh forest of Iran were identified which were mostly of the white-rot type, with one species in each of the brown and soft rot categories. Moreover, the results revealed the sequence data of ITS-rDNA as a useful marker to delimit the fungal species obtained in this study especially those belonging to the genus Xylaria.

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## REFERENCES

**Abrego, N.; Halme, P.; Purhonen, J.; Ovaskainen, O. 2016.** Fruit body based inventories in wood-inhabiting fungi: should we replicate in space or time? *Fungal Ecol* 20: 225-232. https://doi.org/10.1016/j.funeco.2016.01.007

**Aghajani, H. 2012.** Study on the oak (*Quercus castaneifolia*) and Hornbeam (*Carpinus betulus*) decaying macro fungi in mixed Oak-Hornbeam forest community in kheyroud Forest, North of Iran. M.Sc. thesis, Department of Forestry and forest economics. Faculty of Natural resources. University of Tehran, Iran. 95p.

**Aghajani, H.; Marvie Mohadjer, M.R.; Asef, M.R.; Shirvany, A. 2013.** The relationship between abundance of wood macrofungi on Chestnut-leave Oak (*Quercus castaneifolia* C.A.M.) and Hornbeam (*Carpinus betulus* L.) and physiographic factors (Case study: Kheyroud forest, Noshahr). *Journal of Natural Environment IJNRR* 66(1): 1-12. https://doi.org/10.22059/jne.2013.35399

**Aghajani, H.; Marvie Mohadjer, M.R.; Asef, M.R.; Shirvany, A. 2014.** The relationship between wood-decay fungi abundance and some morphological features of Hornbeam (Case study: Kheyroud forest, Noshahr). *Iranian Journal of Forest and Range Protection Research* 12 (1): 55-65. https://doi.org/10.22092/ijfrpr.2014.106538

**Aghajani, H.; Marvie Mohadjer, M.R.; Asef, M.R.; Shirvany, A. 2016 .** Abundance of wood decay macrofungi in forest ecosystems with different management histories in the Kheyroud forest, Nowshahr, northern Iran. *J Forest Res-JPN* 1(4): 295-305. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=606182

**Aghajani, H.; Mohadjer, M.R.M.; Bari, E.; Ohno, K.M.; Shirvany, A.; Asef, M.R. 2017.** Assessing the Biodiversity of Wood Decay Fungi in Northern Forests of Iran. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 88(4): 1463-1469. https://doi.org/10.1007/s40011-017-0887-3

**Aghajani, H.; Bari, E.; Bahmhani, M; Miha, H.; Tajick Ghanbary, M.A.; Nicholas, D.D.; Zahedian, E. 2018.** Influence of Relative Humidity and Temperature on Cultivation of *Pleurotus* species. *Maderas-Cienc Tecnol* 20(4): 571-578. https://doi.org/10.4067/S0718-221X2018005004501

**Bari, E. 2014.** Potential of biological degradation of oriental beech wood by the white-rot fungus *Pleurotus ostreatus* and the effects on mechanical and chemical properties there of and its comparison with standard the white-rot fungus *Trametes versicolor*. MSc. thesis, Sari Agriculture and Natural Resources University, Sari, Iran.

**Bari, E.; Nazarnezhad, N.; Kazemi, S.M.; Tajick Ghanbary, M.A.; Mohebby, B.; Schmidt, O.; Clausen, C.A. 2015a.** Comparison of degradation capabilities of the white rot fungi *Pleurotus ostreatus* and *Trametes versicolor*. *Int Biodeterior Biodegr* 104: 231-237. https://doi.org/10.1016/j.ibiod.2015.03.033

**Bari, E.; Oladi, R.; Schmidt, O.; Clausen, C.A.; Ohno, K.; Nicholas, D.D.; Ghodskhah Daryaei, M.; Karim, M. 2015b.** Influenceof xylem ray integrity and degree of polymerization on bending strength of beech wood decayed by *Pleurotus ostreatus* and *Trametes versicolor*. *Int Biodeter Biodegr* 104: 299-306. https://doi.org/10.1016/j.ibiod.2015.06.019

**Bari, E.; Schmidt, O.; Oladi, R. 2015c.** A histological investigation of Oriental beech wood decayed by *Pleurotus ostreatus* and *Trametes versicolor*. *For Path* 45: 349-357. https://doi.org/10.1111/efp.12174

**Bari, E.; Taghiyari, H.R.; Mohebby, B.; Clausen, C.A.; Schmidt, O.; Vaseghi, M.J. 2015d.** Mechanical properties and chemical composition of beech wood exposed for 30 and 120 days to white-rot fungi. *Holzforschung* 69: 587-593. https://doi.org/10.1111/efp.12174

**Bari, E.; Taghiyari, H.R.; Naji, H.R.; Schmidt, O.; Ohno, M.K.; Clausen, C.A.; Bakar, E.S. 2016.** Assessing the destructive behavior of two white-rot fungi on beech wood. *Int Biodeterior Biodegr* 114: 129-140. https://doi.org/10.1016/j.ibiod.2016.06.010

**Bari, E.; Karim, M.; Oladi, R.; Tajick Ghanbary, M.A.; Ghodskhah Daryaei, M.; Schmidt, O.; Benz, J.P.; Emaminasab, M. 2017.** A comparison between decay patterns of the white-rot fungus *Pleurotus ostreatus* in chestnut–leaved oak (*Quercus castaneifolia*) shows predominantly simultaneous attack both in vivo and in vitro. *For Path* 47(4): e12338. https://doi.org/10.1111/efp.12338

**Bari, E.; Mohebby, B.; Naji, H.R.; Oladi, R.; Yilgor, N.; Nazarnezhad, N.; Ohno, K.; Nicholas, D.D. 2018.** Monitoring the cell wall characteristics of degraded beech wood by white-rot fungi: anatomical, chemical, and photochemical study. *Maderas-Cienc Tecnol* 20(1): 35-56. http://dx.doi.org/10.4067/S0718-221X2018005001401

**Bari, E.; Daniel, G.; Yilgor, N.; Kim, J.S.; Tajick Ghanbary, M.A.; Singh, A.P.; Ribera, J. 2020.** Comparison of the decay behavior of two white-rot fungi in relation to wood type and exposure conditions. *Microorganisms* 8: 1931. https://doi.org/10.3390/microorganisms8121931

**Bari E.; Bari, E.; Pizzi, A.; Schmidt, O.; Amirou, S.; Tajick Ghanbary, M.A.; Humar, M. 2021.** Differentiation of fungal destructive behaviour of wood by the white-Rot fungus *Fomes fomentarius* by MALDI-TOF Mass Spectrometry. *J Renew Mater* 9(3): 381-397. https://doi.org/10.32604/jrm.2021.015288

**Cao, Y.; Wu, S.H.; Dai, Y.C. 2012.** Species clarification of the prize medicinal *Ganoderma* mushroom " Lingzhi". *Fungal divers* 56: 49-62. https://doi.org/10.1007/s13225-012-0178-5

**Edgar, R.C. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32: 1792-1797. https://doi.org/10.1093/nar/gkh340

**Halme, P.; Kotiaho, J.S. 2012.**The importance of timing and number of surveys in fungal biodiversity research. *Biodivers Conserv* 21: 205-219. https://doi.org/10.1007/s10531-011-0176-z

**Hashemi, S.A.; Zare, R.; Khodaparast, K.; Elahinia, A. 2015.** Phylogenetic analysis of Xylaria species in North of Iran based on ITS sequence data. 2nd Iranian Mycological Congress, University of Tehran, Karaj, Iran.

**ImageJ. 2020.** Imaging Procesing and Analysis in Java**.** https://imagej.nih.gov/ij/download.html

**Kubartova, A.; Ottosson, E.; Dahlberg, A.; Stenlid, J. 2012.** Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. *Mol Ecol* 21: 4514-4532. https://doi.org/10.1111/j.1365-294X.2012.05723.x

**Kauserud, H.; Högberg, B.; Knudsen, H.; Elbornes, S.A.; Schumacher, T. 2004.** Molecular phylogenetics suggest a North American link between the anthropogenic dry rot fungus *Serpula lacrymans* and its wild relative *S. himantioides*. *Mol Ecol* 13: 3137-3146. https://doi.org/10.1111/j.1365-294X.2004.02307.x

**Lombard, F.F.; Chamuris, G.P. 1990.** Basidiomycetes. In *Identification manual for fungi from utility poles in the eastern United States*. Wang, C.J.K.; Zabel, R.A. (eds). Am Type Cult Coll: Rockville, United States. ISBN 0930009312, 9780930009311. pp. 21-104.

**Luley, C.J. 2005.** *Wood decay fungi common to urban living trees in the Northeast and central United States*. Urnan Forestry LLC: Naples, United States. ISBN 978-0976712916

**Marvie Mohadjer, M.R. 2011.** *Silvicultur*e. 3nd ed. University of Tehran Press: Tehran, Iran.

**Mullis, K.B.; Faloona, F.A. 1987.** Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Meth Enzymol* 155: 35-50. https://doi.org/10.1016/0076-6879(87)55023-6

**Nobles, M.K. 1965.** Identification of cultures of wood-inhabiting hymenomycetes. *Can J Bot* 43: 1097-1139. https://doi.org/10.1139/b65-126

**Nylander, J.A.A. 2004.** Mr Modeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. https://www.researchgate.net/publication/285805344_MrModeltest_V2_Program_Distributed_by_the_Author#fullTextFileContent https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1276217

**Pandey, K.K.; Pitman, A.J. 2003.** FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *Int Biodeter Biodegr* 52(3): 151-160. https://doi.org/10.1016/S0964-8305(03)00052-0

**Potyralska, A.; Schmidt O.; Moreth, U.; Lakomy, P.; Siwecki, R. 2002.** rDNA-ITS sequence of *Armillaria* species and a specific primer for *A. mellea*. *For Genet* 9: 119-123.

**Pristaš, P.; Kvasnová, S.; Gáperová, S.; Gašparcová, T.; Gáper, J.; Lehtijärvi, A. 2017.** Application of MALDI-TOF mass spectrometry for in vitro identification of wood decay polypores. *For Pathol* 47(5): e12352. https://doi.org/10.1111/efp.12352

**Rambaut, A. 2009.** Fig Tree v1.3.1. http://tree.bio.ed.ac.uk/software/figtree/

**Ronquist, F.; Huelsenbeck, J.P. 2003**. Mr Bayes 3: Bayesian phylogenetic inference under mixed mod-

els. *J Bioinform* 19: 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

**Ryvarden, L.; Gilbertson, R.L. 1993.** *European polypores*. Part 1. Oslo: Fungiflora, Norway. https://www.cabdirect.org/cabdirect/abstract/19952305306

**Ryvarden, L.; Gilbertson, R.L. 1994.** *European polypores*. Part 2. Oslo: Fungiflora, Norway. https://www.cabi.org/ISC/abstract/19951000185

**Schmidt, O. 2006.** *Wood and tree fungi. Biology, damage, protection, and use*. Springer: Berlin. 334p.

**Schmidt, O.; Kebernik, U. 1989**. Characterization and identification of the dry rot fungus *Serpula lacrymans* by polyacrylamide gel electrophoresis. *Holzforschung* 43: 195-198. https://doi.org/10.1515/hfsg.1989.43.3.195

**Schmidt, O.; Moreth, U. 1998a.** Characterization of indoor rot fungi by RAPD analysis. *Holzforschung* 52: 229-233. https://doi.org/10.1515/hfsg.1998.52.3.229

**Schmidt, O.; Moreth, U. 1998b.** Detection of the dry rot fungus *Serpula lacrymans* by amplified ribosomal DNA restriction analysis. *Int Research Group Wood Preserv* 10245. 8p.

**Schmidt, O.; Moreth, U. 1999**. rDNA-ITS sequence of *Serpulalacrymans* and other important indoor rot fungi and taxon-specific priming PCR for their detection. *Int Research Group Wood Preserv* 10298. 10p.

**Schmidt, O.; Moreth, U. 2000.** Species-specific PCR primers in the rDNA-ITS region as a diagnostic tool for *Serpula lacrymans*. *Mycol Research* 104: 69-72. https://doi.org/10.1017/S0953756299001562

**Schmidt, O.; Moreth, U. 2002.** Data bank of rDNA-ITS sequences from building-rot fungi for their identification. *Wood Sci Technol* 36: 429-433. https://doi.org/10.1007/s00226-002-0152-6

**Schmidt, O.; Gaiser, O.; Dujesiefken, D. 2012.** Molecular identification of decay fungi in the wood of urban trees. *Eur J For Res* 131: 885-891. https://doi.org/10.1007/s10342-011-0562-9

**Schmidt, O.; Kallow, W. 2005.** Differentiation of indoor wood decay fungi with MALDI-TOF mass spectrometry. *Holzforschung* 59: 374-377. https://doi.org/10.1515/HF.2005.062

**Staden, R. 1996.** The Staden sequence analysis package. *Mol Biotechnol* 5:233. https://doi.org/10.1007/BF02900361

**Stalpers, J.A. 1978.** Identification of wood-inhabiting aphyllophorales in pure culture. *Studies in Mycology* 16. https://www.studiesinmycology.org/index.php/issue/18-studies-in-mycology-no-16

**Stewart, J.E.; Kim, M.S.; Klopfenstein, N.B. 2018.** Molecular genetic approaches toward understanding forest-associated fungi and their interactive roles within forest ecosystems. *Curr Forestry Rep* 4: 72. http://doi.org/10.1007/s40725-018-0076-5

**Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. 2013.** MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 30: 2725-2729. http://doi.org/10.1093/molbev/mst197

**Terho, M.; Hantula, J.; Hallaksela, A.M. 2007.** Occurrence and decay patterns of common wood-decay fungi in hazardous trees felled in the Helsinki City. *For Pathol* 37(6): 420-432. https://doi.org/10.1111/j.1439-0329.2007.00518.x

**Vigrow, A.; Palfreyman, J.W.; King, B. 1991.** On the identity of certain isolates of *Serpula lacrymans*. *Holzforschung* 45:153-154. https://doi.org/10.1515/HF.2000.038

**White, T.J.; Bruns, T.; Lee, S.; Taylor, J. 1990.** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*. Innis, M.A; Gelfand, D.H; Sninsky, J.J; White, T.J. (eds). Academic Press: San Diego, California, United States. pp. 315-322.

**Young, R.J. 1982.** *Introduction to Forest Science*. John Wiley & sons. ISBN 978-1111308391