CHARACTERIZATIONS OF TREE-DECAY FUNGI BY MOLECULAR AND MORPHOLOGICAL INVESTIGATIONS IN ANIRANIAN ALAM DARDEH 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-seqencing.

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
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 bodies collected 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.

| Family          | Species          | Collection ID       | GenBank Accession No. | Source * |
|-----------------|------------------|---------------------|-----------------------|----------|
| Xylariaceae     | X. bayeri       | CBS 12620           | KX206484              | CBS      |
|                 | X. bayeri       | RP432               | KX096386              | DSMZ     |
|                 | X. bayeri       | STF 132             | MG853884              | STF       |
|                 | X. bayeri       | CBS 14763           | KX900007              | CBS      |
|                 | X. bayeri       | CBS 14673           | AF163038              | CBS      |
|                 | X. bayeri       | NTB 0122            | KJ204698              | NTB      |
|                 | X. bayeri       | STF 133             | MG853885              | STF       |
|                 | X. polyphora    | STF 146             | MG853886              | STF       |
|                 | X. polyphora    | NIB 1456            | KX204440              | NIB      |
|                 | X. polyphora    | IPO 9780            | AF163041              | IPO      |
|                 | X. polyphora    | CBS 59072           | M860951               | CBS      |
|                 | X. polyphora    | STF 118             | MG853808              | STF       |
| Agaricales Physalacriaceae | A. melo | AFTOL-ID:1488       | Y970861               | AFTOL    |
|                 | A. melo         | STF 110             | MG853859              | STF       |
| Pleurotaceae    | P. brunneus     | CBS 4203            | KX499378              | TENN     |
|                 | P. polyononis   | ZBS 12021           | KX922728              | CBS      |
|                 | P. polyononis   | STF 123             | MG853875              | STF       |
|                 | P. ostrearia    | CBS 6689            | KX453400              | TENN     |
|                 | P. ostrearia    | STF 185             | JQ834752              | ZBS MSU  |
|                 | P. ostrearia    | CBS 23947           | KX429309              | CBS      |
|                 | P. ostrearia    | STF 122             | MG853874              | STF       |
| Strophariaceae  | P. aurellla     | TENN 01741          | MB855317              | TENN     |
|                 | P. aurellla     | CBS 118              | MB866660              | CBS      |
|                 | P. aurellla     | STF 121             | MG853872              | STF       |
| Schizophyllaceae | S. commune     | SCA 121              | AJ168962              | SCA      |
|                 | S. commune     | SCA 221              | FJ732688              | BCC      |
| Polyporaceae    | S. commune     | STF 127             | MG853879              | STF       |
|                 | S. adspersin    | CBS 355              | EU162853              | CBS      |
|                 | S. adspersin    | HSBU 20094           | MG276154              | BFC      |
|                 | S. adspersin    | JN222318             | MG853862              | STF       |
|                 | S. adspersin    | GKA 120829           | MG853865              | STF       |
|                 | S. adspersin    | SFC156010-23       | KJ643556              | STF       |
|                 | S. adspersin    | STF 114             | MG853863              | STF       |
|                 | S. adspersin    | STF 116             | MG853865              | STF       |
|                 | S. adspersin    | AM209773             | KX922049              | STF       |
|                 | S. adspersin    | KMA60597             | KX841476              | BFC      |
|                 | S. adspersin    | SYMC 097            | JX009053              | SYMC     |
|                 | S. adspersin    | TEM 102             | HS869941              | TEM      |
|                 | S. adspersin    | SYMC 1005           | KX189779              | BFC      |
|                 | S. adspersin    | Cm1059              | KX189779              | BFC      |
|                 | S. adspersin    | CBS 69594            | JX640981              | CBS      |
|                 | S. adspersin    | KS128               | MG853880              | STF       |
|                 | S. gibbosa      | STF 121             | MG853881              | STF       |
|                 | S. gibbosa      | CBS 28430            | MG855141              | CBS      |
|                 | S. fruticola    | BRFM-FRA-1094       | JX645100              | BRFM     |
|                 | S. fruticola    | CBS 28273            | MG866885              | CBS      |
|                 | S. fruticola    | STF 130             | MG853882              | STF       |
|                 | S. versicolor   | BRFM-FRA-1219       | JX645098              | BRFM     |
|                 | S. versicolor   | CBS 12153           | DQ44179               | CBS      |
|                 | S. versicolor   | STF 151             | MG853883              | STF       |
| Lepiocraeae     | I. lactea       | CBS 431             | KX695652              | CBS      |
|                 | I. lactea       | KUC36004            | JX295071              | KUC      |
| Moniliaceae     | P. radiata      | SFC 2015010-23     | MF459006              | SFC      |
|                 | P. radiata      | ATCC 64658           | FJ664656              | ATCC     |
|                 | P. radiata      | STF 126             | MG853870              | STF       |
| Lasiosphaeriaceae | I. sulphurina | KATAN-3              | EU840607              | SUN      |
|                 | I. sulphurina   | SVPP4                | EU840599              | SUN      |
|                 | I. sulphurina   | EBT-111              | FL405564              | FMR      |
|                 | I. sulphurina   | STF 119             | MG853869              | STF       |
| Neosphaeriaceae | N. ditissima     | CBS 37988            | AJ60625               | TENN     |
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* AFTOL: Assembling the Fungal Tree of Life, USA; ATCC: American Type Culture Collection, Manassas, VA, USA; BCC: Biorepositories Technology Unit, National Center for Genetic Engineering and Biotechnology, Thailand; BFBC: Institute of Microbiology, Beijing Forestry University, China; BRFM: Banque de Reseaux Fongiques de Marseille, France; CBS: Wursterdijk Fungal Biodiversity Institute, Netherlands; CFMR: Center for Forest Mycology Research, Wisconsin, USA; CMU-Unix: Mycological Herbarium of the Mycological Center of the University of Bologna, Italy; CRA-PA: 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; DMSZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IIE: 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 Herbarium collection, South Korea; NAAS: National Academy of Agricultural Science, Microbiology, South Korea; NCSL: 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 Alamardeh 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.) Donk | Carpinus betulus/standing | Jan-16 | WR | 7 |
| Ganoderma adspersum Donk | Quercus castaneifolia/standing | Nov-16 | WR | 12 |
| Ganoderma applanatum (Pers.) Pat. | Quercus castaneifolia/standing | Oct-17 | WR | 5 |
| 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.) D. M. Martin | Carpinus betulus/dead standing | Nov-14 | WR | 2 |
| Laetiporus sulphureus (Bull.) Murrill | Quercus castaneifolia/standing | Nov-14 | BR | 2 |
| Plebilia radiata Fr. | Quercus castaneifolia/fallen | Jun-14 | WR | 1 |
| Pholota 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 betellosa (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 |
| Trametes versicolor (L.) Lloyd | Carpinus betulus/fallen | Jun-16 | WR | 5 |
| Xylariabephyxylon (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. appianatum, 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 belonging 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* (AF-TOL-ID 1522).
Figure 5: ITS phylogeny of different fungal taxa belonging 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* (AF-TOL-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 identified using 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 ascomycetous and 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.
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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 fasiculare, Crepidotus sp., Pluteus sp., Coprinus sp., G. applanatum 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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