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# Molecular identification of some wild medicinal macrofungi from Northern Iran

**Alimadadi N[1], Pourianfar HR[2], Amoozegar MA[1,3*], Zabihi SS[1], Mahdizadeh V[1] and Shahzadeh Fazeli SA[1]**

*[1] Microorganisms Bank, Iranian Biological Resource Center (IBRC), Academic Center for Education, Culture and Research (ACECR)-Tehran, Iran*
*[2] Industrial Fungi Biotechnology Research Department, Research Institute for Industrial Biotechnology, Academic Center for Education, Culture and Research (ACECR)- Khorasan Razavi, P.O.Box: 91775-1376, Mashhad, Iran*
*[3] Extremophiles Laboratory, Department of Microbiology, Faculty of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Iran*

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**Abstract**

In last decades, macrofungi have attracted increasing attention because of their valuable nutritional and medicinal properties. In this study, a total of 180 macrofungal samples were collected from forests in Mazandaran province, Iran. The dominant orders were Polyporales (51%) and Agaricales (35%). Pure mycelial cultures were successfully obtained from 91 collected samples. Regarding morphological data, 47 isolates were selected for molecular identification based on internal transcribed spacer region (ITS) sequence analysis. The results showed that the 38 macrofungal isolates were belonging to 22 species, 19 genera, 10 families and 5 orders. Most of the macrofungi (47%) were identified as *Trametes* species and *Ganoderma* species. Three isolates identified as *Hohenbuehelia* species, *Polyporellus brumalis* and *Ceriporia lacerata* were records as a new to the Iran fungal flora. This study increases the knowledge on Iranian macrofungal diversity and facilitates future genetic and biotechnological investigations on these macrofungi.

**Key words** – Internal transcribed spacer region – Iran – Macrofungi – Mazandaran province

## Introduction

Macrofungi are defined as fungi that produce fruiting bodies visible without the aid of a microscope (Redhead 1997). They are extremely diverse in different climate and geographical regions in the world. It is estimated that there are 150,000-160,000 marofungal species present on the earth, among them 16,000 species have been described till date (Hawksworth 2012, Wasser 2010). The macrofungi play a vital role as decomposers, parasites, and symbionts and diet sources in ecosystems (Redhead 1997). In addition, several macrofungi have been used as a source of food and medicine for thousands of years in different civilizations (Money 2016). In last decades, the beneficial property of macrofungi has gained increasing attention by scientific community. Fruiting bodies and mycelia of macrofungi are rich in compounds with nutritional and/or medicinal properties (Cheung 2010, Reis et al. 2017). They are excellent sources of fiber, protein, essential oils, vitamins, minerals and biologically active metabolites such as phenolic compounds, terpenoids,

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Corresponding Author: Mohammad Ali Amoozegar – e-mail – amoozegar@ibrc.ir, amoozegar@ut.ac.ir

polysaccharides, lectins, steroids, lipids, peptides and glycoproteins (El Enshasy & Hatti-Kaul 2013, Rathore et al. 2017, Roncero-Ramos & Delgado-Andrade 2017). Consequently, more than 100 medicinal functions have been attributed to macrofungi including antimicrobial, antiviral, anticancer, antioxidant, immune-modulatory, immune-suppressive, anti-allergic, hepato-protective, anti-diabetic, anti-cholesterol and detoxification activities (Roncero-Ramos & Delgado-Andrade 2017, Roupas et al. 2012, Wasser 2014). Several macrofungal compounds have been subjected to clinical trials and are used extensively in Asia to treat various diseases (Wasser 2014). Moreover, macrofungi have applications as biocontrol agents and in cosmetics (Sivanandhan et al. 2017, Taofiq et al. 2016).

Accurate identification of macrofungi is important for evaluate their diversity and applications. However, there are many reports on biodiversity and biological activity of wild macrofungi without proper authentication (Wasser 2014). Morphological characterization is common method for identification of macrofungi but it is difficult, time consuming and may lack precision in differentiating closely related species. Molecular techniques are fast and reliable and extensively are used for identification of microorganisms (Bisen et al. 2012). Among DNA barcodes, the nuclear ribosomal internal transcribed spacer (ITS) region has proved to have the highest probability of successful identification for the broadest range of fungi including macrofungi (Dentinger et al. 2011, Schoch et al. 2012).

Mazandaran province in the north of Iran is located between Albourz Mountains and Caspian Sea. Heavy to moderate rainfall, mild temperature, and dense vegetation in the forests of this region provide suitable conditions for growth of diverse fungal flora. A number of reports have been published on diversity of macrofungi in this region but most of them are based on morphological characterization (Asef & Etemad 2016, Borhani et al. 2010, Keypour et al. 2014) and still little data is available in the literature (Rezaeian et al. 2016). However, considering the importance of native populations of macrofungi in biomedical research, breeding programs and biodiversity studies, it is essentially required to preserve the samples alive under standard conditions to maintain their original properties. Therefore, this study was conducted with the aim of collection, mycelial culture and molecular identification of wild macrofungi, with emphasis on medicinal ones, from Mazandaran province.

## Materials & Methods

### Collection of macrofungi

Macrofungal specimens were collected from forests in Mazandaran province during the rainy seasons (spring and autumn) between 2015 and 2016. Detailed site information regarding location and elevation are provided in Table 1. The morphological and ecological characteristics of the macrofungi were recorded and photographed in the field. All specimens were labeled, placed in paper bags, and transferred to the laboratory for further study. Initial identification of the macrofungi carried out based on the macroscopic and microscopic characteristics using key provided by Moser (1983), Ryvarden (1991) and Ryvarden & Melo (2014).

### Mycelial culture

Using aseptic tissue culture technique, small cut pieces of fruiting bodies of the macrofungi were inoculated on malt extract agar (2% malt extract, 2% agar) and compost extract agar media supplemented with 0.02% chloramphenicol and incubated under dark condition for two weeks at 25 °C. Compost extract agar was prepared as detailed by Masoumi et al. (2015). Pure cultures were obtained by inoculation of 1 $cm^2$ disks from the leading edges of the mycelia on potato dextrose agar and incubation at the same condition mentioned above. The culture of all identified macrofungi were deposited at Microorganisms Bank of Iranian Biological Resource Center (IBRC-M), Iran.

### DNA extraction

For DNA extraction, the pure mycelial cultures were grown in a liquid medium (0.5% yeast extract, 1% peptone and 2% glucose) for 4-7 days at 25 °C. DNA extracted from a small clump of

the mycelia using the salting-out protocol described by Saba et al. (2016). The quality of DNA was analyzed by agarose gel (1%) electrophoresis stained with ethidium bromide. DNA samples were stored at −20 °C until use.

**ITS region sequencing and phylogenetic analysis**

The sequences of the internal transcribed spacer (ITS) regions (including 5.8S rDNA) determined from polymerase chain reaction (PCR) products amplified from the DNA samples using the primers ITS1 and ITS4 (White et al. 1990). DNA sequencing was carried out using Sanger (dideoxy) method with the mentioned primers. The sequences were assembled using ChromasPro software version 1.5. The sequences were compared pairwise using Basic Local Alignment Search Tool (BLAST) and aligned all sequences (retrieved from GenBank and CBS databases) using the CLUSTAL W (Thompson et al. 1994). Phylogenetic trees were reconstructed using the neighbour-joining algorithm of MEGA 7.0.21 (Kumar et al. 2016). Confidence levels of the clades were estimated from bootstrap analysis based on 1000 replications (Felsenstein 1985).

**Results**

A total number of 180 macrofungal specimens were collected from forests in Mazandaran province. Based on initial morphological characterization, the macrofungi belonged under 2 phyla, 6 orders, 21 families and 38 genera (Table 1). Most of the macrofungi (97%, 175 samples) were classified under the phylum Basidiomycota, whereas others (3%, 6 samples) belonged to the phylum Ascomycota. The dominant orders were Polyporales (51%) and Agaricales (35%). Other macrofungi were distributed in the orders viz. Russulales, Hymenochaetales, Auriculariales, Xylariales and Pezizales. The genera *Trametes* (27%) and *Ganoderma* (21%) were frequent macrofungi among the collected samples.

Pure mycelial cultures were successfully obtained from 91 collected macrofungi, while other specimens did not form mycelia or their cultures were contaminated by other fungi or bacteria. Forty-seven isolates were selected for molecular identification (Table 1) and their ITS region (including 5.8S rRNA gene) were amplified and sequenced. The PCR amplification using primers ITS1 and ITS4 obtained about 550-700 bps DNA fragments. The acquired nucleotide sequences were deposited in the NCBI database and were used for BLAST search. The accession number and the BLAST analysis results were presented in Table 2. Phylogenetic analyses were carried out for the ITS sequence of each isolate and closely related species to confirm species determination (data not shown except for isolates from *Polyporaceae*; Fig. 1). The results corresponded to morphological identification of the samples. It was shown that 38 isolates were basidiomycetous macrofungi belonging to 22 species, 19 genera, 10 families and 4 orders. The families included *Polyporaceae*, *Irpicaceae*, *Phanerochaetaceae*, *Agaricaceae*, *Psathyrellaceae*, *Physalacriaceae*, *Pleurotaceae*, *Strophariaceae*, *Stereaceae* and *Auriculariaceae*. Most of the macrofungi (21 isolates) were identified as members of *Polyporaceae* including 10 and 8 isolates from *Trametes* and *Ganoderma* genera, respectively. Nine isolates were identified as ascomycetous microfungi and were considered as contamination of the macrofungal samples. It is found that two isolates GPS 002 and GPS 208 may be representatives of two novel taxa in *Xylariaceae* and Pleosporales, respectively.

In the phylogenetic tree constructed by ITS sequences of 21 isolates from *Polyporaceae* family (Fig. 1), the isolates were located in 4 distinct clades with high bootstrap values, corresponding the genera *Ganoderma*, *Trametes*, *Fomes* and *Polyporellus*.

In *Ganoderma* clade, 6 isolates located at the same position with an authentic strain of *G. adspersum*. The isolates can be divided into three groups based on ITS region sequence: (1) Bozchaft2, GPS 017 and GPS 047; (2) GPS 037 and GPS 038; and (3) GPS 052. ITS sequence of isolate GPS 052 differs from the sequences of group 1 and 2 by two and one nucleotide substitutions, respectively. Two other isolates in this clade were not identified at species level. The isolates were positioned at the same cluster with the authentic strains of *G. lucidum*, *G. tsugae* and *G. oregonense*. The ITS sequences of the isolates were different in four positions.

In *Trametes* clade, it was shown that 10 isolates were conspecific with one of *T. versicolor*, *T. gibbosa* or *T. hirsuta* species. Among *T. versicolor* isolates, the sequence of GPS 107 differs from two other isolates by one nucleotide substitution. Two isolates of *T. gibbosa* showed one nucleotide variation. ITS sequences of three isolates of *T. hirsuta* were identical but different from the sequence of isolate GPS 119 by two nucleotides.

Among the remaining isolates of *Polyporaceae* family, two isolates were clustered with *F. fomentarius* and one other isolate was closely related to *P. brumalis*. ITS sequence of two isolates of *F. fomentarius* was different by one nucleotide substitution. *Fomes fomentarius* is a species complex containing four distinct clades. It seems that our isolate is more related to Chinese and South European clades but it was located at separate position from the clades.

**Table 1** Sampling data and the results of initial morphological identification of the collected macrofungi.

| **Forest** | **Location*** | **Date of sampling** | **Macrofungi genera (Morphologic identification)** | **Selected isolates for molecular identification** |
|---|---|---|---|---|
| Nur | N36 34 E51 48, 24-55 m | 9/23/2015 | *Coprinellus*, *Ganoderma*, *Lactarius*, *Mycena*, *Pleurotus*, *Psathyrella*, *Trametes*, *Volvariella*, *Xerula* | Nur 2, Nur 8, Nur 9, Nur 10 |
| Royan | N36 51 E51 94, 221-243 m | 9/24/2015 | *Daedaleopsis*, *Irpex*, *Lactarius*, *Lenzites*, *Leucoagaricus*, *Omphalotus*, *Pleurotus* | Royan 6 |
| Bozchaft | N36 38 E52 76, 186-192 m | 4/18/2016 | *Ganoderma*, *Trametes* | Bozchaft 2 |
| Darab Kola | N36 29 E53 18, 661-860 m | 4/20/2016 | *Collybia*, *Crepidotus*, *Cyclocybe*, *Daldinia*, *Exidia*, *Fomes*, *Ganoderma*, *Hypholoma*, *Lenzites*, *Pleurotus*, *Schizophyllum*, *Stereum*, *Trametes* | Darabkola 1, Darabkola 4, Darabkola 8, Darabkola 18, Darabkola 21 |
| Neka | N36 33 E53 23, 190-941 m | 4/21/2016 | *Donkia*, *Ganoderma*, *Helvella*, *Pleurotus*, *Trametes*, *Xylaria* | Neka 24D, Neka 29-1 |
| Nur | N36 33 E52 05, 17-18 m | 5/24/2016 | *Coprinus*, *Crepidotus*, *Ganoderma*, *Mycena*, *Trametes* | GPS 002, GPS 005, GPS 016, GPS 017 |
| Kashpel-Lavij | N36 23 E52 02, 331-692 m | 5/24/2016 | *Trametes* | GPS 022 |
| Si Sangan | N36 34 E51 48, 55-58 m | 5/25/2016 | *Ceriporia*, *Crepidotus*, *Pleurotus*, *Trametes* | GPS 029 |
| Dalkhani | N36 82 E50 66, 650-801 m | 5/26/2016 | *Ganoderma*, *Trametes*, *Trichaptum* | GPS 037, GPS 038, GPS 042, GPS 047, GPS 052, GPS 057 |
| Chalus | N36 62 E51 42, 257 m | 5/27/2016 | *Trametes* | GPS 063 |
| Abbas Abad-Kelardasht | N36 38 E51 06 375-416 m | 10/26/2016 | *Ganoderma*, *Hypholoma*, *Macrolepiota*, *Trametes* | GPS 101, GPS 106, GPS 107 |
| Safa-Rud | N36 39 E53 35, 382-567 m | 10/27/2016 | *Crepidotus*, *Fomes*, *Ganoderma*, *Lycoperdon*, *Pholiota*, *Pleurotus*, *Schizophyllum*, *Trametes*, *Xylaria* | GPS 119, GPS 122, GPS 128, GPS 131, GPS 142, GPS 146, GPS 158 |
| Zirab-Lajim | N36 14 E53 03, 856-882 m | 11/10/2016 | *Armillaria*, *Bjerkandera*, *Ganoderma*, *Hypholoma*, *Polyporellus*, *Stropharia*, *Trametes* | GPS 167, GPS 172, GPS 173, GPS 177, GPS 179, GPS 180 |
| Abbas Abad-Behshahr | N36 39 E53 35, 382-511 m | 11/11/2016 | *Armillaria*, *Ganoderma*, *Hericium*, *Hohenbuehelia*, *Pleurotus*, *Xerula* | GPS 186, GPS 188, GPS 196, GPS 197, GPS 208 |

**Table 2** The results of BLAST search for ITS region sequences of selected isolates and the related GenBank and IBRC-M accession numbers.

| Isolate | IBRC-M Acc. No. | Genbank Acc. No. | Taxon | Closest hit in BLAST search | Genbank Acc. No. | Identity |
|---|---|---|---|---|---|---|
| Bozchaft 2 | 30422 | MK050589 | *Ganoderma* sp. | *G. adspersum* | MG066632 | 100% |
| Darabkola 1 | 30421 | MK050606 | *Stereum* sp. | *S. armeniacum* | MH862626 | 100% |
| | | | | *S. hirsutum* | KY628654 | |
| Darabkola 4 | 30428 | MK050610 | *Trametes hirsuta* | *T. hirsuta* | JX501305 | 100% |
| Darabkola 18 | 30354 | MK050586 | *Exidia* sp. * | *E. glandulosa* | MF161201 | 99% |
| Darabkola 21 | 30427 | MK050587 | *Fomes fomentarius* | *F. fomentarius* | LT629714 | 99% |
| Nur 8 | 30409 | MK050607 | *Trametes gibbosa* | *T. gibbosa* | MH277950 | 100% |
| Nur 9 | 30430 | MK050584 | *Coprinellus* sp. * | *C. disseminatus* | JN159560 | 99% |
| Nur 10 | 30431 | MK050605 | *Psathyrella* sp. * | *P. candolleana* | MH856032 | 99% |
| Neka 24D | 30310 | MK050585 | *Donkia pulcherrima* | *D. pulcherrima* | LC378994 | 99% |
| GPS 005 | 30434 | MK050600 | *Irpex* sp. * | *Irpex* sp. | MH267976 | 99% |
| | | | | *I. lacteus* | MG554250 | |
| GPS 017 | 30436 | MK050590 | *Ganoderma* sp. * | *G. adspersum* | MG279153 | 100% |
| GPS 022 | 30424 | MK050608 | *Trametes gibbosa* | *T. gibbosa* | MF161242 | 100% |
| GPS 029 | 30437 | MK050583 | *Ceriporia* sp. * | *Ceriporia* sp. | KJ832049 | 100% |
| | | | | *C. lacerata* | KP135024 | |
| GPS 037 | 30403 | MK050591 | *Ganoderma* sp. * | *G. adspersum* | JN588579 | 99% |
| GPS 038 | 30405 | MK050592 | *Ganoderma* sp. * | *G. adspersum* | JN588579 | 99% |
| GPS 042 | 30438 | MK050611 | *Trametes hirsuta* | *T. hirsuta* | JX501305 | 100% |
| GPS 047 | 30439 | MK050593 | *Ganoderma adspersum* | *G. adspersum* | JN222417 | 100% |
| GPS 052 | 30407 | MK050594 | *Ganoderma* sp. | *G. adspersum* | JN588579 | 99% |
| GPS 057 | 30440 | MK050617 | *Trichaptum* sp. | *T. biforme* | FJ755247 | 99% |
| GPS 063 | 30441 | MK050609 | *Trametes gibbosa* | *T. gibbosa* | KM373239 | 100% |
| GPS 101 | 30327 | MK050602 | *Macrolepiota* sp. | *M. konradii* | JQ683125 | 99% |
| | | | | *M. gracilenta* | JQ683122 | |
| GPS 106 | 30442 | MK050597 | *Hypholoma fasciculare* | *H. fasciculare* | JQ685719 | 99% |
| GPS 107 | 30404 | MK050614 | *Trametes* sp. * | *T. versicolor* | MF475935 | 100% |
| GPS 119 | 30408 | MK050612 | *Trametes* sp. * | *T. hirsuta* | KC589148 | 99% |
| GPS 122 | 30443 | MK050615 | *Trametes* sp. * | *T. versicolor* | MH320563 | 99% |
| GPS 128 | 30444 | MK050613 | *Trametes versicolor* | *T. versicolor* | MF782818 | 100% |

**Table 2** Continued.

| Isolate | IBRC-M Acc. No. | Genbank Acc. No. | Taxon | Closest hit in BLAST search | Genbank Acc. No. | Identity |
|---|---|---|---|---|---|---|
| GPS 131 | 30313 | MK050588 | *Fomes* sp. * | *F. fomentarius* | KP641149 | 99% |
| GPS 142 | 30316 | MK050603 | *Pholiota* sp. | *P. aurivella* | KT355030 | 98% |
| GPS 146 | 30411 | MK050599 | *Irpex lacteus* | *I. lacteus* | MH301114 | 100% |
| GPS 158 | 30318 | MK050601 | *Lycoperdon pyriforme* | *L. pyriforme* | KP454030 | 100% |
| GPS 167 | 30423 | MK050947 | *Bjerkandera adusta* | *B. adusta* | MH857085 | 100% |
| GPS 172 | 30426 | MK050948 | *Ganoderma* sp. | *G. lucidum* | MG911000 | 99% |
| GPS 173 | 30400 | MK050582 | *Armillaria* sp. * | *A. mellea* | AF163583 | 99% |
| GPS 177 | 30355 | MK050598 | *Hypholoma* sp. * | *H. fasciculare* | KX449406 | 99% |
| GPS 179 | 30399 | MK050616 | *Trametes* sp. * | *T. hirsuta* | MH910542 | 100% |
| GPS 180 | 30324 | MK050604 | *Polyporellus* sp. * | *P. brumalis* | KP283490 | 99% |
| GPS 186 | 30334 | MK050595 | *Ganoderma* sp. | *G. lucidum* | MG911000 | 99% |
| GPS 196 | 30342 | MK050596 | *Hohenbuehelia* sp. | *H. auriscalpium*<br>*H. petaloides* | LN714552<br>KT388021 | 99% |
| **Ascomycetous microfungi (contamination)** | | | | | | |
| Darabkola 8 | 30429 | MK050620 | *Nemania* sp. * | *N. serpens* | HM123484 | 100% |
| Nur 2 | 30425 | MK050622 | | | | 100% |
| Neka 29-1 | 30432 | MK050621 | *Neopestalotiopsis* sp. | *Neopestalotiopsis* sp. | MG649986 | 100% |
| GPS 016 | 30435 | MK050623 | | | | 99% |
| Royan 6 | 30433 | MK050619 | *Didymosphaeria* sp. | | | |
| GPS 188 | 30311 | MK050618 | (*Paraconiothyrium brasiliense*) | *P. brasiliense* | MH532510 | 99% |
| GPS 197 | 30416 | MK050625 | *Pochonia* sp. * | *P. chlamydosporia* | AB709845 | 100% |
| GPS 002 | 30446 | MK050626 | *Xylariaceae* sp. | *Xylariaceae* sp. | JQ761922 | 98% |
| GPS 208 | 30445 | MK050624 | *Pleosporales* sp. | *Uncultured Pezizomycotina* | KT581743 | 98% |

* Species delineation using phylogenetic analyses for the strains were matched to the closest hit in BLAST search. However, the results cautiously were not submitted.

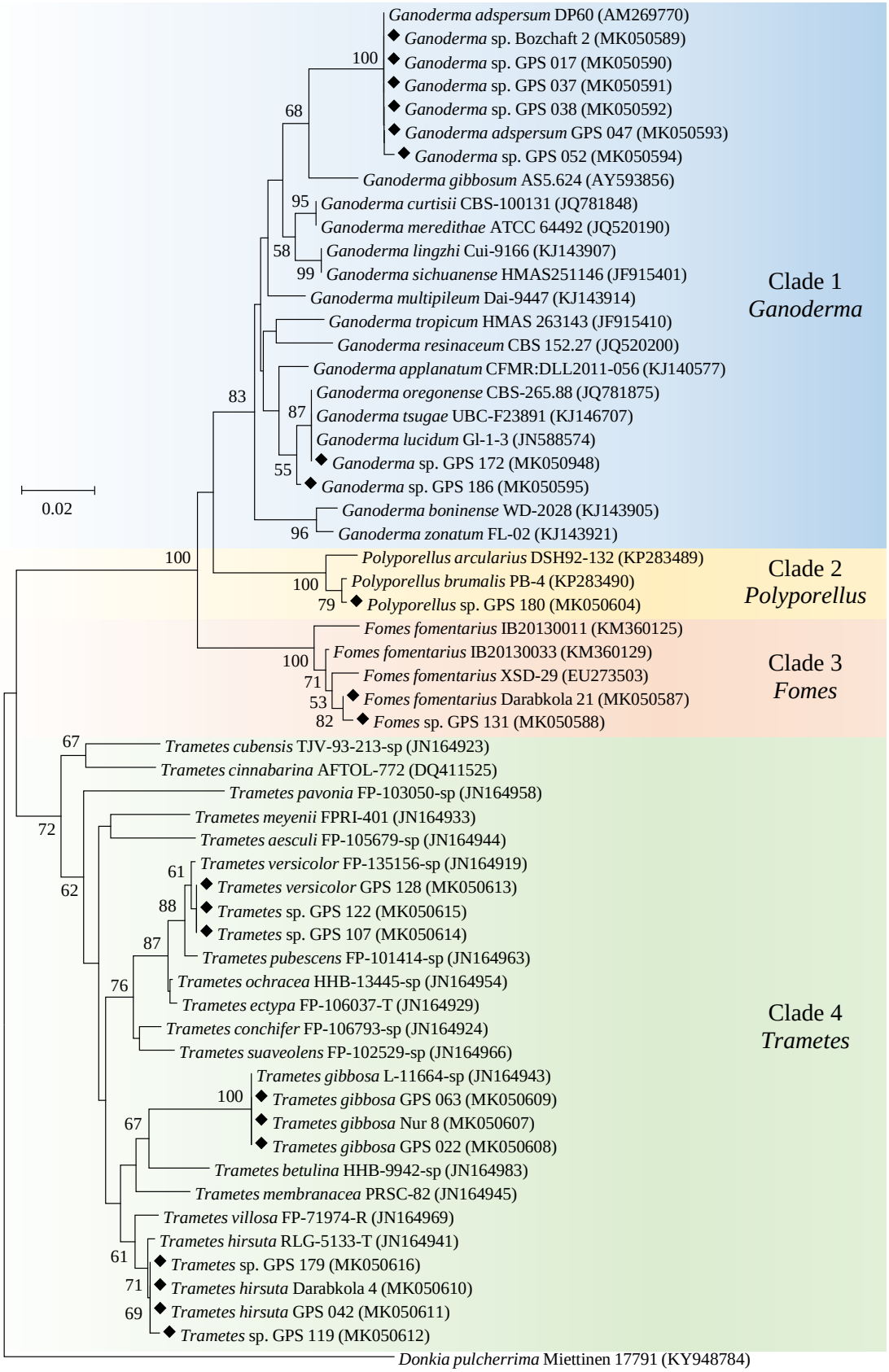


**Fig. 1** – Phylogenetic tree based on ITS region (including 5.8S rRNA gene) sequence showing the position of macrofungal strains in *Polyporaceae* family among the related species. The phylogram was constructed from evolutionary distance data with Kimura's two-parameter correction, using the neighbor-joining method. *Donkia pulcherrima* Miettinen 17791 was the designated outgroup for the analysis. Bootstrap values above 50% (based on 1000 replicates) are given at branch points. Bar, 0.02 substitutions per nucleotide position.

**Discussion**

Study on diversity of wild populations of edible and medicinal macrofungi provides opportunities to apply their beneficial properties. There are several reports on collection and morphological characterization of wild macrofungi from Iran, especially Northern Iran (Amoopour et al. 2016, Asef 2008, Asef & Muradov 2012, Borhani et al. 2010, Karim et al. 2012, 2013, Moradali et al. 2007, Olfati et al. 2009). In this study, 38 macrofungi genera were identified based on initial morphological features from Mazandaran province. In a checklist of Iranian non-gilled/non-gasteroid hymenomycetes published by Ghobad-Nejhad & Hallenberg (2012), Mazandaran province had the second highest diversity in Iran including 214 species from 128 genera. Borhani et al. (2010) reported 100 species from 57 genera from this province. Most of collected genera of this study have been reported previously from Mazandaran province; among them two genera *Hohenbuehelia* and *Polyporellus* can be considered as new records for Iran. The identity of the samples was confirmed by ITS sequencing and phylogenetic analysis. The isolate GPS 196 was collected from Abbas Abad forest and showed highest ITS sequence similarity (99%) to *H. auriscalpium* and *H. petaloides*. Further study using multi-locus sequence analysis is needed for identification of the isolate at species level. The isolate GPS 180 was collected from a forest in Zirab-Lajim region. The ITS sequence of the isolated was 99% similar to the related sequence from *P. brumalis* strains and located at the same clade with them in the phylogenetic tree (Fig. 1). *Polyporellus brumalis* have been mentioned in excluded taxa of the checklist presented by Ghobad-Nejhad & Hallenberg (2012). In addition, isolate GPS 029 was closely related to *Ceriporia lacerata* based on NCBI and CBS BLAST searches and phylogenetic analysis of the ITS sequence. The species have not been reported previously from Iran in the available literatures.

Morphological and ecological characterization have been used by most of studies on biodiversity of macrofungi in Iran (Amoopour et al. 2016, Asef 2008, Asef & Mudarov 2012, Borhani et al. 2010, Ghobad-Nejhad & Langer 2016, Karim et al. 2012, 2013, Olfati et al. 2009). There are few reports for molecular identification of wild populations of Iranian macrofungi using ITS region sequence analysis (Ghobad-Nejhad & Langer 2016, Rezaeian et al. 2015, Tajalli et al. 2015). Therefore, there is a lack of molecular data for Iranian macrofungi in available databases. In this study, 38 ITS region sequences from 22 species were obtained and deposited on GenBank. ITS sequencing and phylogenetic analysis were successfully applied for taxon determination. However, further studies using other gene markers is required for species identification at species level in some cases (Table 2). For example, translation elongation factor 1-α (*tef1-α*) and the second largest subunit of RNA polymerase II (*rpb2*) loci have been applied for classification of *Ganoderma* species (Matheny et al. 2007, Jargalmaa et al. 2017). It was demonstrated that the combination of morphological and molecular (ideally multi-locus) analysis can resolve the problems related to taxonomy of fungi (Jung et al. 2014, Jargalmaa et al. 2017).

Macrofungi have great importance in food, medicine and cosmetics. Several beneficial properties have been reported for most of species studied in this study (Agrawal et al. 2017, Ahmad 2018, Cespedes et al. 2015, Doskocila et al. 2016, Dresch et al. 2015, Dyakov et al. 2011, El Enshasy & Hatti-Kaul 2013, Reis et al. 2017, Tang et al. 2018, Tel-Çayan et al. 2015, Yang et al. 2013, Yin et al. 2014). Some isolates were closely related to medicinally important macrofungi including *Ganoderma lucidum*, *G. adspersum*, *Trametes versicolor*, *T. hirsuta*, *T. gibbosa*, *Fomes fomentarius*, *Armillaria mellea*, *Irpex lacteus* and *Stereum hirsutum*. Pure mycelial cultures of the macrofungi are available in IBRC-M culture collection and can be used to evaluate their potential applications. Collection and morphological identification of some *Ganoderma* species from Iran have been reported by Moradali et al. (2007) and Keypour et al. (2014). They reported that Iranian *Ganoderma* species include *G. applanatum*, *G. adspersum*, *G. colossus*, *G. lucidum*, *G. resinaceum*, *G. tsugae* and *G. manoutchehrii*. Heydarian & Hatamian-Zarmi (2016) identified an Iranian *G. lucidum* strain (HA2012-001) using ITS sequencing for the first time. The strain was isolated from Dohezar forest in Mazandaran province. The ITS sequence of the strain (GenBank accession number KX765192) differs from our strains GPS 172 and GPS 186 by two and four nucleotide substitutions, respectively. Therefore, they are different strains. ITS sequence variation was also observed among our strains

belonging to *G. adspersum, T. versicolor, T. hirsuta T. gibbosa* and *I. lacteus* strains isolated from different forests in Mazandaran province. These data indicate great diversity of macrofungi in Northern Iran. Beside biodiversity evaluations, precise identification by molecular methods is important from applied point of view due to species-specific and strain-specific production of bioactive compounds by medicinal macrofungi.

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