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# Morphological and molecular characterization of *Bjerkandera adusta* (Meruliaceae), a new addition to macromycota of Iraq

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**Abstract:** *Bjerkandera adusta* plays an important role in decaying lignin and human-derived pollutants and has a promising role in the textile industry (i.e. in decolorization of synthetic dyes). Reports on the Meruliaceae family and its genus *Bjerkandera* are not available from Iraq. During fieldwork in Salahadin Governorate (north-central Iraq) in 2019, *B. adusta* was collected and identified as a new addition to the macromycota of Iraq, based on morphological characters and molecular analysis. A detailed morphological description of this species and its habit, habitats, and distribution are provided and sequence data and phylogenetic tree are presented. This is the second report on the identification of macrofungi from Iraq, based on morphological and molecular characterization.

## 1. Introduction.

*Bjerkandera adusta* (Willd.) P. Karst. (also known as a smoky bracket), the type species of the genus *Bjerkandera* P. Karst, in the family Meruliaceae (Agaricomycetes: Basidiomycota), is a cosmopolitan polypore characterized by the gray or ashy to the black hymenial surface, monomitic hyphal system and abundant clamp-connections [1,2]. This white-rot fungus plays an imported role in the nutrient recycling by decaying deadwood of hardwoods and in bioremediation due to its ability to degrade human-derived pollutants such as polycyclic hydrocarbons including industrial dyes [3-7]. In medicine, *B. adusta* is an important fungus associated with lung inflammation [8, 9]. However, reports on the family Meruliaceae and its genus *Bjercandera* are not available from Iraq.

Salahadin Governorate (north-central Iraq) is one of the largest agricultural Governorates in Iraq (24.363 $Km^2$ geographical area). This Governorate is rich in vegetation, including many tree species (such as *Pinus* sp., *Populus* sp., *Salix* sp., and a few fruit tree species) and diverse species of shrubs and herbs. This vegetation richness provides suitable habitats for the growth of different macro-fungal species. This governorate also shows topographic variation, from steppe to desert in the south to foothills in the north. Despite this phytogeographic importance, information on macrofungi from this Governorate as well as from other parts of Iraq is still very limited. However, most of the studies carried out on the identification and classification of macrofungi in Iraq were only based on the morphological characters which often resulted in the misidentification of the collected fungal samples. Thus, molecular analysis is a necessary tool for the accurate taxonomy of these fungi. In this study, *B. adusta* was collected and identified as a new confirmed record to Iraqi macromycota, based on both morphological characteristics and molecular analysis.

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## 2. Materials and methods.

### *2.1. Sampling and Study sites.*

Samples were collected from, Tikrit (34°43'51.1"N 43°38'48.0"E, elevation 137 m) and Al-Alam (34°42'29.4"N 43°41'43.7"E, elevation 96 m) provinces in Salahadin Governorate, during Jan.-Feb. 2019. The samples were photographed in natural habitats and laboratories. Habitat (substrate/host) and habit (growth forms) were recorded. Macroscopic and microscopic features were reported. Melzer's reagent, 3% KOH, and cotton blue in lactophenol were used for microscopy. Identification of the samples was performed according to literature, keys, and monographs [1, 2, 4, 10, 11]. The identified specimens were deposited in NCBI GenBank (MW254998.1) and in the Biology Dept., College of Education for Pure Sciences, Tikrit University, Iraq.

### *2.2. Molecular identification.*

#### *2.2.1.DNA Extraction.*

Powdered *B. adusta* fruiting bodies were used to extract DNA, using the ZR Fungal/Yeast/Bacterial DNA MiniPrep kit protocol (ZYMO/ USA) according to the manufacturer's instructions.

#### *2.2.2. The ribosomal ITS region.*

The internal transcribed spacer ITS (Integrated DNA technologies /USA) region of the rDNA was amplified by PCR with tow universal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [12]. Maxime™ PCR PreMix Kit (i-Taq) (USA) was utilized for PCR reactions following the manufactures instructions. PCR amplification conditions were as follows: 94 °C for 3min initiating denaturation, 35 cycles including 94 °C for 45s, 52 °C for 1min and 72 °C for 1min, and a final extension at 72 °C for 7min. Amplification products were sent for sequencing in MacroGen Ltd. (Korea).

#### *2.2.3. Bioinformatic analysis.*

For species identification, the nucleotide sequence data were compared with sequences available at the National Center for Biotechnology Information (NCBI) internet database (GenBank). The ribosomal ITS sequence of the *Bjerkandera* species was used for the Basic Local Alignment Search Tool (BLAST) algorithm analysis at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequence alignments of the *Bjerkandera* sp. sequence and sequences of the species, got from the GenBank (Table 1), were performed with molecular evolutionary genetics analysis Mega-X Program version 10.0.5 [13], were align by Clustal W provided in the Phylogeny.fr software [14]. The identification between sequences was determined as a percentage of species sequence.

**Table 1. The molecular identity of the macrofungi samples used in this study.**

| No. | Sample code | Species | Region | % Identity | Accession No. |
|---|---|---|---|---|---|
| 1 | A1 | *Bjerkandera adusta* | Iraq | 100% | MW254998.1 |
| 2 | A2 | *Polypolares* sp. | France | 97.60% | JQ312134.1 |

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| | | | | | |
|---|---|---|---|---|---|
| 3 | A3 | *Bjerkandera fumosa* | Poland | 97.94% | JX891534.1 |
| 4 | A4 | *Bjerkandera adusta* | Iran | 98.28% | MF497751.1 |
| 5 | A5 | *Bjerkandera adusta* | France | 98.42% | GU731546.1 |
| 6 | A6 | *Bjerkandera adusta* | United Kingdom | 97.93% | KP794071.1 |
| 7 | A7 | *Bjerkandera adusta* | Italy | 97.75% | KJ093490.1 |
| 8 | A8 | *Bjerkandera adusta* | China | 97.43% | MK829590.1 |

## 3. Results

Based on morphological features and sequence data, *B. adusta* was identified as a new record for Iraqi macromycota. Morphological and molecular characterization, illustrations, and comments are given as follows:

*3.1. Macroscopic features.*

Caps annual, sessile, semi-circular, flat, forming overlapping thin brackets and fuse laterally, 2-12 cm wide, 2-6cm deep, up to 0.8 cm thick; Upper (sterile) surface: finely haired to velvety, grey, grey-brown, slightly zoned; margin irregular, white when young then brown to grey-brown, sharp at age. Lower (fertile or hymenial) surface: porous, white, becoming grey to blackish at age, darkening when injured, 4-6 pores/mm, pores angular, rounded to oval, up to 250 µm wide, tube layer 2-5 mm thick. Flesh thin; whitish, elastic to leathery. Oder fungal when fresh, not distinctive when dried; surfaces negative in KOH (Fig.1 and 2).


**Figure 1.** *B. adusta* in nature. A-C, on Salix sp., D, E. on Pinus sp., F. on Populus sp.

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**Figure 2.** *B. adusta* in the Lab. (from Pinus tree-Fig.1. D&E). A, B. upper surface in fresh specimens, D. specimen in B. when dried, E. the grey lower surface, F. lower surface with black bruising.

*3.2. Microscopic features.*

Basidia 10.0-12.5 × 5.0-6.0 µm, 4-spored; spores 5.0-6.0 × 2.5-3 µm, ellipsoid, cylindrical, smooth, hyaline in KOH, inamyloid: Cystidia absent; Hyphal system monomitic, hyphae, smooth, sometimes branched, with clamp connections, 2-5 µm wide. Habit and habitat; Saprotrophic, forming overlapping brackets on dead wood of broad and needle-leaved trees of *Pinus* sp., *Populus* sp. and *Salix* sp. It may cover a wide area (more than 50 cm wide) on the trunk as in the burned trunk of willow (*Salix* sp.). It occurs on living trees of *Populus* and *Salix* as well, but not on living Pinus trees (Fig.3). On living trees starts saprotrophic then parasitic, causing white rot. Season: year-round often in autumn and winter. Locality: Almuhzam in Tikrit and Aldafsha and Effry in Al-Alam localities.

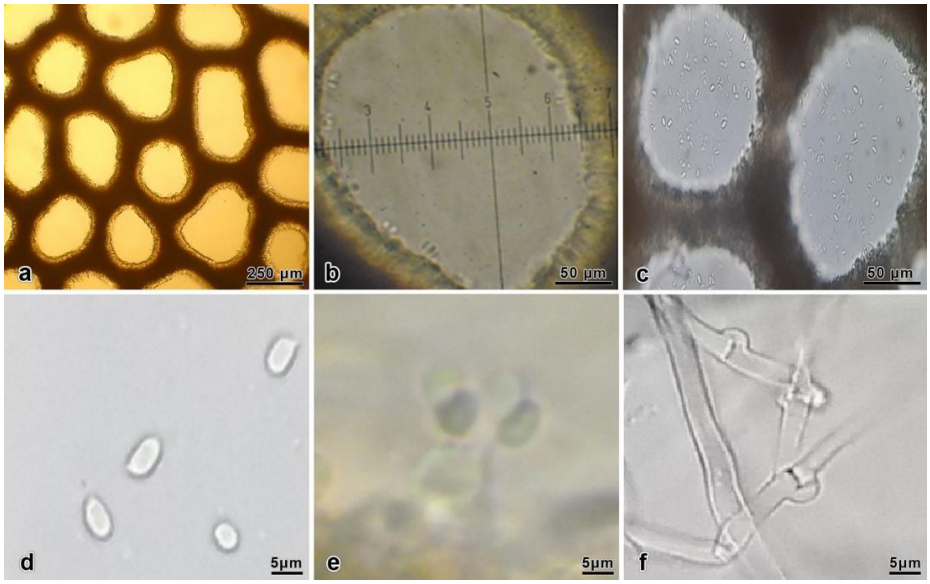


**Figure 3.** *B. adusta*. A. pores in the lower surface, B. pore magnified, C. pores with spores, D. spores, E. basidium, F. Clamp – connections.

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*3.3. Molecular Analyses of rDNA-ITS fungal species.*

In addition to the phenotypic and microscopically description to confirm the diagnosis of the fungal species used in this study, it has been molecularly studied with the PCR amplification of the rDNA-ITS domain using ITS1 and ITS4 Primers created DNA segment 650 bp (Fig. 4), and the rDNA-ITS sequences of the A1 sample gave high similarity score (97.43% - 98.42%) with available sequence alignment from BLAST analysis at the NCBI website and sample were then deposited online in the GenBank (www.ncbi.nlm.nih.gov) with the accession number MW254998.1.

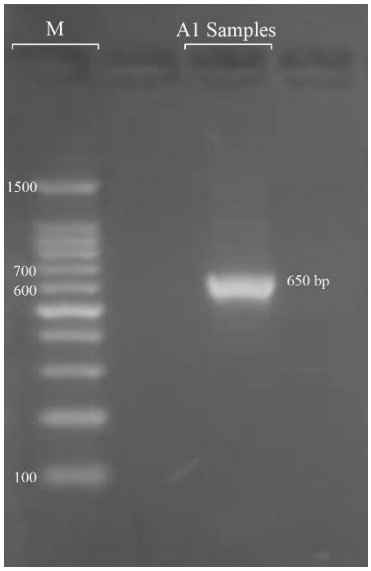


**Figure 4.** Electrogram of PCR product showing the band size (650 bp) of ITS segment of the sample studied, the product was electrophoresis on 2% agarose at 5 volt/cm$^2$, 1x TBE buffer for 1:30 hours, DNA ladder (100) lane M., and lane A1 samples indicate to *B. adusta*.

The phylogenetic analysis of Iraqi samples was based on the phylogenetic tree (Fig. 5), sample A1 was identified as *B. adusta* (MW254998.1), sample A2 was *Polypolares* sp. (JQ312134.1) and sample A3(JX891534.1) was *B. fumosa*, on the other hand, samples A4-A8 were identified as *B. adusta* (MF497751.1, GU731546.1, KP794071.1, KJ093490.1, and MK829590.1) respectively. Tow separated clustered clades appeared in the phylogenetic tree, which identified with the specimens of the same species presented in the GenBank, sample A1 appeared with samples A3, A4, A5, A6 and A8 as a separated clustered clade, and it showed the highly similar with them, whereas sample A2 and A7 showed in the deferent cluster. The phylogenetic tree showed clear genetic differentiation between the species and analyses revealed for the identification accuracy of sequences available in GenBank that more identified with other *B. adusta* sequences.

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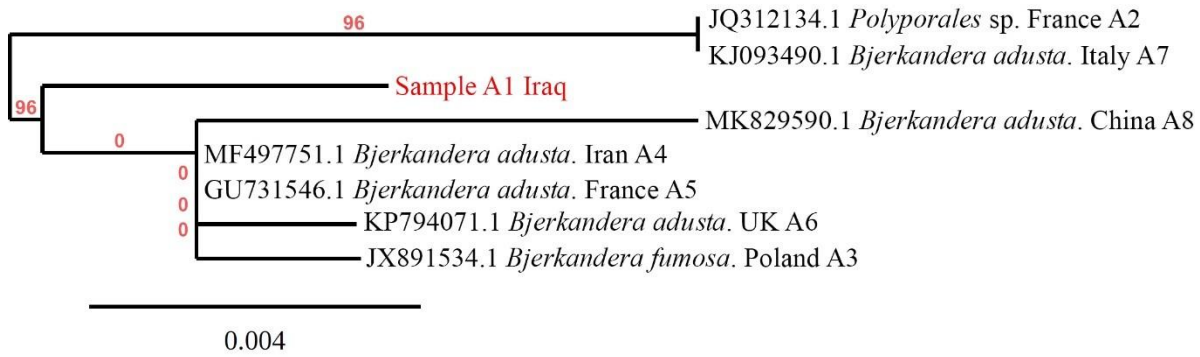


**Figure 5.** Phylogenetic tree showing cluster analysis and the relationship of the Iraqi *Macromycota* collected.

## 4. Discussion

In this study, the family Meruliaceae and its most representative fungus *B. adusta* (the type species of the genus *Bjerkandera*) were reported as new taxa records for the Iraqi macromycota based on morphological characteristics and molecular analysis. *B.adusta* occurs in many parts of the world, like North America [1], Korea [4], China [9], Russia [10], UK [11] Turkey [15], Iran [16], and Tunisia [17].

This white rot polypore plays an important role in nutrient recycling in the forest ecosystems and in bioremediation as well [4]. Several species are similar or closely related to *B. adusta*. *Trametes versicolor* is a similar species but it has a white hymenial surface and its sterile surface is distinctly zoned with various colors. Another closely related, *Bjerkandera fumosa*, caps of *B. adusta* are thinner with darker hymenial surface and occur on both broad and needle-leaved trees (*B. fumosa* occurs only on broad-leaved trees). This paper is the second molecular phylogenetic report on the macrofungi of Iraq, after Al-khesraji [18]. However, sequences of *Bjerkandera* and other macrofungi are crucial for correct identification of the species and their applications. In this regard, few published molecular studies on *Berkandera* spp. are available, see: [2, 4,19, 20].

The inquiry for ITS sequences in GenBank found 60 sequences were labeled as *B. adusta* out of 102 sequences, the remaining 42 sequences were identified as *B. fumosa*, *Polypolares* sp., *Thanatephorus cucumeris*, *Agaricomycetes* sp., *Marasmius cohaerens*, *Basidiomycota* sp., *Rhizoctonia* sp., fungal sp., and uncultured fungus. DNA sequences are a forceful tool to help in species identification, DNA barcoding has gotten famous for species ID since it is simple and direct to utilize, nonetheless, the adequacy of DNA barcoding relies upon open databases having agreeable classification sampling and sequences that are accurately distinguished [21, 22]. The phylogenetic investigation uncovered the position of species distinguished as *Bjerkandera* sp. from Iraq and that the two unique origins show to various species [2], and the results in this study also represent to generally perceived *B. adusta* and *B. fumosa*.

A few researchers unequivocally depicted the trouble recognizing *Bjerkandera* and *Thanatephorous* utilizing DNA sequences, because of the deeply similar sequences of the two

distinct species presented in NCBI [23]. In the past taxonomy the genus *Bjerkandera* was treated as closely related, on the other hand, modern studies of the molecular analysis showed the two genera were independent of each other, and this may indicate that the genus *Bjerkandera* has been neglected but it has many species and must be treated over a wider range to include all species [24]. Therefore, it must be on its classification to include all species and distribution in the world.

## 5. Conclusion

The present study reports *Bjerkandera adusta* as a new confirmed record for the macromycota of Iraq, based on morphological characteristics and molecular analysis. *B. adusta* is the type species of the genus *Bjerkandera* and the most common representative of the family Meruliaceae. However, no previous information is available on this family and its genus *Bjerkandera* from Iraq. So, greater attention is needed to explore various aspects of these two taxa.

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