Molecular identification and phylogeny of *Polyporus plorans*, new record from Iraq

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Abstract

Polyporus plorans is reported for the first-time from Salahadin Governorate, North Central Iraq. Globally, it is considered a rare species. The species is morphologically described based on the macroscopic and microscopic features and molecularly confirmed rely on the nuclear ribosomal internal transcribed spacer region. The species sequence is newly generated and submitted in NCBI. This newly generated sequence is the second deposited ITS sequences worldwide submitted to GenBank. The environmental features for the first recoded species are provided. The species relationship between the similar species and other species is also detected.

Keywords: *Polyporus plorans*, rare species, 2nd deposited ITS sequences bioinformatic analyses

Introduction

The genus *Inonotus* P. Karst (Hymenochaetaceae, Hymenochaetales) includes 101 species distributed around the word. Most of studies on the occurrence and systematics for its species has been carried out for European and North American (Wanger and Fischer, 2002; Dai 2010) [24, 9]. It was erected to house all polypores based on the type of basidiocarps (Dai 2010) [9]. The genus is still a matter of dispute regarding the limits and nomenclature of the species (Ranadive, 2013) [18]. It has very important species with medicinal fungi and some of them forest pathogens. The *Polyporus plorans* (Pat.) Sacc. & D. Sacc, synonymy *Inonotus plorans* (Pat.) Bond. & Sing., is considered a rare species related to this genus. The specimen has been collected and recorded for the present only from Morocco (Wanger & Fischer, 2002) [24] poroid Hymenochaetales and Algeria in North Africa on *Salix* sp. and *Populus* sp. (Ryvarden, 2005) [20]. The species was also collected from the North West Iran on the bark of Juglans regia (Nejhad, & Kotiranta, 2008) [16]. However, the species was previously listed and described by Pegler (1964) [17] based on type examination of Algeria collection. The species was also listed with Indian checklist of family Polyporaceae during the preceding 40 years (Ranadive, 2013) [18]. Genetically, there are four sequences in the NCBI database related to this species in the worldwide. Three of them are related to the large subunit (LSU) from China and Germany and the last one is sequenced the internal transcribed spacer region (ITS) region from China. The wild macrofungi, the result of sexual reproduction, have been hardly studied in Iraq for scientifical and environmental reasons (Al Anbagi *et al*., 2022) [1]. However, recently many studies have been documented and most of them were for the first-time recording. Few species related to Ascomycota were detected from the middle south of Iraq (Al anbagi, 2014) [3]. Many species of Basidiomycota have been collected from the north part of Iraq (e.g. Suliaman, 2017) [21] where this part received substantial rainfall between October and April with annual precipitation 700 -1000 mm with temperature 8-25 ºC (Al Anbagi *et al*., 2022) [1]. In Salah ad-Din province (north-central Iraq), several important species have been described and some of them were genetically confirmed linked to coprinoid fungi such as *Coprinellus*, *Parasola*, *Psathyrella* (Al-Khesraji *et al*., 2017a, Al-Khesraji, 2017b, AL-Khesraji *et al*., 2018) [5, 21, 4]. In the present study, *Polyporus plorans* is recorded and described for the first time. Its identification was genetically confirmed and phylogenetically detected its relationships using sequence data.

Materials and method

Collected samples

The sample was collected from Al-Sharqat district in Salah Al-Din Governorate. The sample was photographed and described on its natural substrate. Other related information such as the habit, habitat, type of natural substrate, and collection date, and color was also recorded in the natural field. The basidiocarp was collected from the tree trunk with clean knife, placed in the paper bag, and marked. Later, the sample was transferred to the laboratory in the College of Science, University of Tikrit, for future process.

Morphological description

The phenotypic characteristics of the fruiting body were documented (Johnsy *et al*., 2011) [13]. These included the dimensions, shape, color, surface texture of the fruiting body, and the presence or absence of a stem. The microscopic characteristics of fungal tissue were recorded after preparing slides stained with Cotton Blue in lactophenol. The slides were microscopically examined and measured under 40X magnification using a light microscopy. The microscopic

features included description the shape, color, and dimensions of the spores and the type of basidia and cystidia. The current scientific name and taxonomic position of the species follow the recent edition of authors of fungal names available in the international Index Fungorum website (www.indexfungorum.org).

DNA extraction and sequencing

The macromorphological identification of the current species was confirmed using DNA amplifications and sequencings of the ITS region. Small pieces of fresh basidium were used for DNA extraction using the E.Z N. A. Fungal DNA Mini-Preps Kit. (Omega Bio-tek) following the manufacturer's guidelines. The extracted DNA were stored in at -20°C until processed for PCR amplification. The extracted DNA was amplified using ITS1 and ITS4 primers (White *et al*., 1990) [25]. The PCR amplification was achieved in a total volume of 25 µl. This volume was prepared by adding 4 µl DNA, 2µl PCR PreMix (Intron, Korea), and 2µl of each primer (10 pmol). Finally, the deionized distilled water was added to complete the total volume. The thermal cycling conditions were as follows: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 52°C for 1 min, and 72 °C for 1min, followed by 72 °C for 7 min using a Thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The amplified product was visualized and sized on 1% agarose gel via electrophoresis stained with Red Safe (Al Anbagi *et al*., 2019) [2]. Later, the product was sequenced using the Sanger sequencing method (Microgen, Seoul, Korea).

Raw sequence data was manually edited and spontaneously assembled in the Geneious program version 9.1.8 (Biomatters Ltd., Newark, New Jersey). The identity of represented sequence was verified using the Basic Local Alignment Search Tool (BLAST) available at the National Center for National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov/genbank/) and compared with other fungal nucleotide sequences. The newly generated sequence was submitted to GenBank.

Phylogenetic analyses

The phylogenetic tree was constructed to elucidate the genetic interspecies relationship after being retrieved from GenBank other related ITS sequences (Table. 1). The selected sequences were aligned using MAFFT (Thompson *et al*., 1994) [22]. The maximum likelihood (ML) analysis in Gen software was performed for to the phylogenetic analysis. ML tree was constructed using RAxML. The bootstrap analysis was run with 1000 replicates. The tree was rooted with the outgroups with genera that considered as closed to the genus *Polypore* (Wanger & Fischer, 2002; Larsson, *et al*., 2006) [24, 15].

Table 1: Species, country and GenBank accession number of sequences used in this study

Species name	Country	GenBank accession no.	References
Inonotus plorans	China	MZ484607	Wu *et al*., 2022 [26]
Inonotus cuticularis	China	MZ484527	Wu *et al*., 2022 [26]
Inonotus hispidus	Morocco	KF446596	Zhou *et al*., 2014 [27]
Inonotus hispidus	UK	AY558602	Jeong *et al*.,2005 [11]
Inonotus quercustris	Argentina	AY072026	Gottlieb *et al*., 2002 [10]
Phellinus igniarius	Czech Republic	GQ383713	Tomsovsky *et al*., 2010 [23]
Fomitiporella sinica	China	KX181300	Ji *et al*., 2017 [12]

Results and discission

Description of morphological

Macroscopic features

The basidiocarp is semicircle and joined to the substrate by a stalk-like part (Fig.1.A). Pilus was tough large light weight when dray with upper surface finely tomentose and. Pilus sized 35 cm wide and 5 cm thick with rounded margin. The cap was yellow with red-brown softy and spongy when flesh. The pore surface is cinnamon to brown becoming dark with pores 2-3 per mm round to angular with deep tubes. The pores exuded water to gelatin drops on its surface (Fig.1.).

Microscopic character

The hyphal system is hyphae clampless, simple septate, tramal hyphae yellow, thin- to thick-walled and, contextual hyphae golden yellow to brown, parallel to subparallel. Basidia are hyaline with projection of four sterigma and attached basidiospores. Basidiospores smooth, ellipsoid, yellow to pale brown, thick walled and sized about 7.5-10 x 5-7.5 µm. The type of Cystidia is pleurocystidia, 7.5-12.5 µm wide (Fig.2).

Habit and habitat

One basidiocarp sample is collected near the cavity of the high part of the living tree trunk, *Populus* sp. The sample was found in the garden that was dominated by *Citrus sinensis* and *Vitis* spp. and *Populus* trees

Season

It was collected in March 2021, Alshurkat / Salahaldin districts.

Edible validity

Not known locally.

Comments

This is the first record in Iraq. The species is rare and has been collected only from Algeria on *Salix* and *Populus* (Ryvarden, 2005) [20] and region of north western China (BaoKai *et al*.,2007) [6] and NW Iran from bark of a cultivated Juglans regia in a private orchard (Ghobad-Nejhad & Kotiranta, 2008) [16].

Species classification

Polyporus plorans (Pat.) Sacc. & D. Sacc., Syll. fungi. (Abellini) 17: 110 (1905)**.**

Synonymy

Inonotus plorans (Pat.) Bondartsev & Singer, *Annls mycol.* 39(1): 56 (1941) Xanthochrous plorans Pat., Bull. Soc. mycol. Fr. 20: 52 (1904).

Position in classification

Hymenochaetaceae, Hymenochaetales, Agaricomycetidae, Agaricomycetes, Basidiomycota, Fungi.

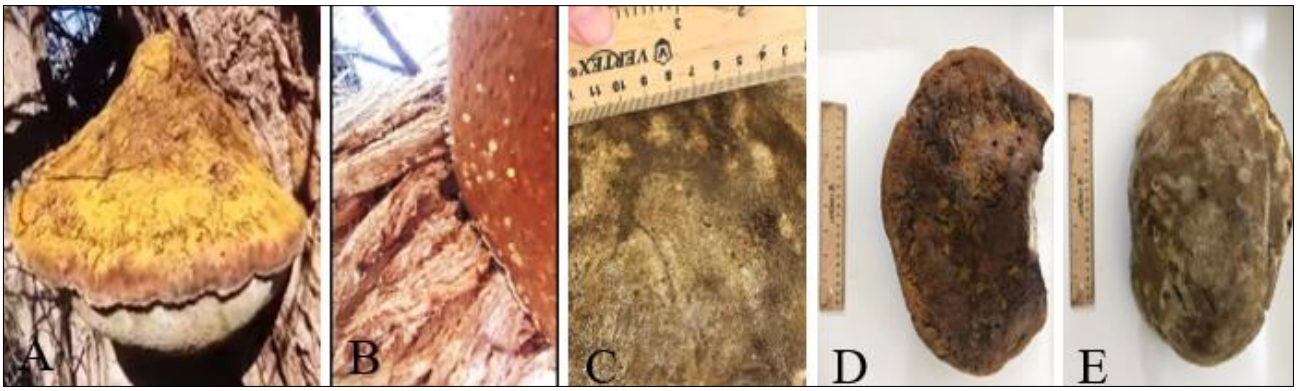

Fig 1: Basidiocarps of *Polyporus plorans* from Iraq. (A, B) basidiocarp in natural habitats, (C) hymenium layer, (D, E) basidiocarp in laboratory

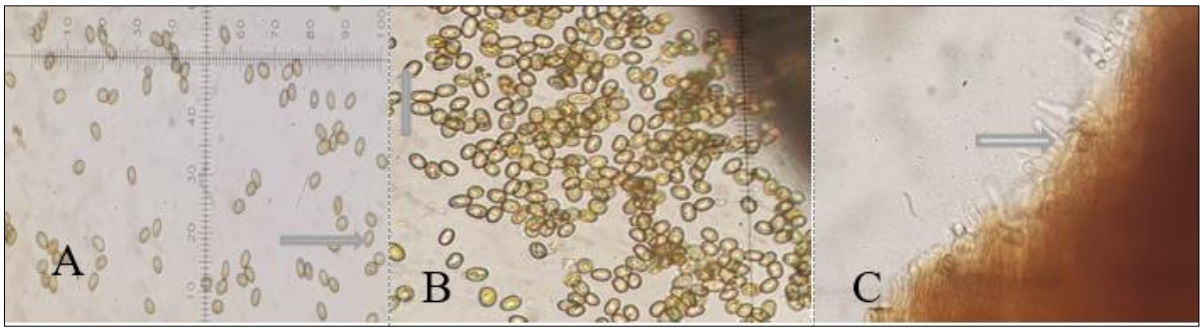

Fig 2: Micrmorphological characters of *Polyporus plorans*. (A) cystidia, (B, C) basidio spores

Molecular identification and phylogeny

The species sequence of the sample was confirmed morphological affinity. The blast results for sequence similarity identified the species as *Polyporus plorans*. The species is related to *Inonotus s. str.*, characterized by annual to perennial basidiomata as an independent genus (Dai, 2010) [9]. The similarity percentage of the newly generated sequence was 98.9% with the specimen *Inonotus plorans* voucher from China that has accession number MZ484607. The sequence of Iraqi species was submitted into GenBank under the accession number OP828561. This newly generated ITS sequence is the second deposited sequences worldwide submitted to GenBank. Globally, there are only four sequences of *P. plorans* in the global GenBank database, one of which is for ITS and the other three are for the LSU region. Three of these sequences are submitted from China and the last one from Germany.

The analysis showed that the newly sampled species from Iraq nested within the *Inonotus* clade. The phylogeny concluded from the ITS dataset, clearly signified that represented species were related to a polyphyletic genus (Fig. 2) supporting previous DNA analyses.

The phylogeny tree showed that *Inonotus* spp. grouped into different clades and *P. plorans* from Iraq was grouped together with *P. plorans* from China based on ITS sequence data. In current phylogeny, *I. cuticularis* clustered outside the grouped *P. plorans* indicated a close relationship with *P. plorans*. The two species are characterized by not having hyphoid setae. The close relationship between *P. plorans* and *I. cuticularis* in current results is similar to the species positions in a tree built using the nLSU data by Ren (2018) [19]. The group of presented species is also next to *I. hispidus* which is hispid and has occasional setae. Morphology, both species have large basidiocarp. However, microscopically *P. plorans* and *I. hispidus* have basidiospores longer than 6 µm with thick-walled spores. The *I. plorans*, *I. cuticularis* and *I. hispidus* were considered to be members of *Inonotus sensu stricto* (Wei Zhou *et al*., 2015) [28]. These produce annual basidiocarps with a monomitic hyphal system (Wei Zhou *et al*., 2015) [28]. With *I. quercustris*, the previous species shared similar colored basidiospores (Ryvarden, 2005; Dai, 2010) [20, 9]. However, the difference grouped between species based on ITS-based phylogeny might be related to other distinctively morphologic features lead to low resolution of ITS region.

In conclusion, the current record species specified that the distribution of this rare species extends to Iraq although the harsh environment of the country. More studies are needed to determine the distribution of this species and other species related to the current genus.

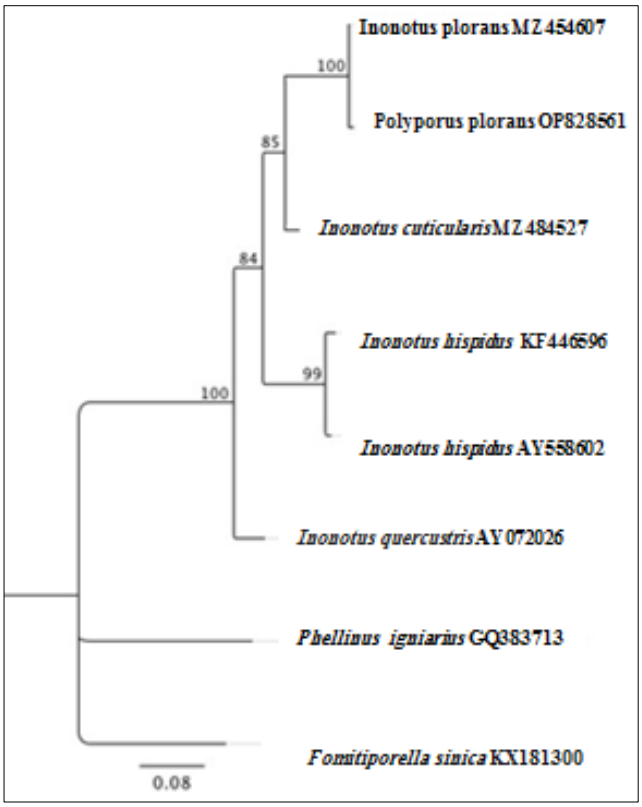

Fig 3: Phylogeny of Polyporus and related genera inferred from ITS data

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