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A new pathogenic polypore on urban trees: The first record of *Rigidoporus ulmarius* (Rigidoporaceae, Hymenochaetales) in Hungary

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RESEARCH ARTICLE

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ABSTRACT

The Giant Elm Bracket (*Rigidoporus ulmarius*) is a widely-distributed necrotrophic polypore species that causes white heart rot in deciduous trees. Despite its recognition as one of the largest species known for forming basidiomata, this perennial polypore had not been documented in Hungary. However, in recent years, two specimens macroscopically resembling this species were collected on old horse chestnut (*Aesculus hippocastanum*) trees from two different places in Hungary by amateur mycologists. In this study, subsequent morphological and molecular-genetic analyses of these fungal samples confirmed their identity as *R. ulmarius*. This study represents the first documented occurrence of this plant pathogenic polypore species in Hungary.

KEYWORDS

Basidiomycota, polypore, ITS, phylogeny, phytopathogenic

INTRODUCTION

The basidiomycete genus *Rigidoporus* Murrill, along with the genera *Bridgeoporus* T.J. Volk, Burds. & Ammirati and *Leucophellinus* Bondartsev & Singer, belongs to the family

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Rigidoporaceae Jülich within the order Hymenochaetales Oberw. (Wang et al., 2023b). The type species of the genus *Rigidoporus* is *Rigidoporus microporus* (Sw.) Overeem, a widely distributed plant pathogenic species in the tropics, which can have significant economic importance in rubber, cocoa, or tea plantations (Oghenekaro et al., 2020; Saidi et al., 2023; Yuan et al., 2023). *Rigidoporus ulmarius* (Sowerby) Imazeki, is also a plant pathogenic polypore species has a cosmopolitan distribution (e.g., Rajchenberg and Robledo, 2013; Ryvarden and Melo, 2014; Ryvarden et al., 2022; Wang et al., 2023a). In Europe, it grows preferably on *Ulmus*, but also reported on other deciduous trees, including popular park and roadside trees in Hungary (e.g., *Aesculus*, *Celtis*, *Fraxinus*, *Platanus*, *Populus*) (Ryvarden and Melo, 2014). The basidiomata of this perennial polypore grows on the lower part of the trunk of mature living trees, and causing a white heart rot determining large cavities inside the host (Bernicchia and Gorjón, 2020).

Despite the description of *Phellinus ellipsoideus* (B.K. Cui & Y.C. Dai) B.K. Cui, Y.C. Dai & Decock (formerly *Fomitiporia ellipsoidea* B.K. Cui & Y.C. Dai), until then *R. ulmarius* was considered the fungus which forms the largest basidiomata in the world (Tribe, 2003; Dai and Cui, 2011). Nonetheless, this distinct and remarkable perennial polypore species had not been documented in Hungary until now. However, in the monitoring of macrofungi in Hungary, citizen science has increasingly played a significant role. Thanks to this, in recent years, I have obtained two mushroom samples from amateur mycologists that macroscopically resemble the basidiocarps of *R. ulmarius* from two different locations. The first fungal specimen was collected in Keszthely, Zala County, while the second one originated from Nagyatád, Somogy County. In both cases, the basidiocarps were found growing at the base of old horse chestnut (*Aesculus hippocastanum*) trees in urban environments. Accordingly, the objective of this study is to determine the identity of these fungal specimens through the use of micromorphological and molecular phylogenetic methods.

MATERIAL AND METHODS

Isolates and morphology

The specimens were deposited in the fungarium of the Hungarian University of Agriculture and Life Sciences. Macromorphological descriptions was based on field notes. Micromorphological data were obtained from dried specimens, which were observed under a Zeiss Axio Imager.A2 light microscope, equipped with AxioVision Release 4.8.2. software. Measurements were done with a 100× oil immersion objective (1000× magnification). Observations of microscopic features as well as measurements were made on slide preparations stained with Melzer's reagent. Spores were measured by cutting sections from the tubes. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range. The following abbreviations were used in the description of the basidiospores: IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid (Melzer's-negative reaction), L = mean spore length, W = mean spore width, Q = variation in the L/W ratios, n = number of measured spores.

Molecular study

Primers ITS1F and ITS4 (Gardes and Bruns, 1993; White et al., 1990) were used to amplify the ITS (internal transcribed spacer) region of the nuclear ribosomal DNA. For the amplification,

the Phire® Plant Direct PCR Kit (Thermo Scientific, USA) was used, following the manufacturer's recommendations. The PCR (polymerase chain reaction) protocols were set according to Papp and Dima (2018). The quality of PCR products was checked in 2% agarose gels. The amplicons were sequenced commercially at the Biological Research Centre (Szeged, Hungary) with the same primers used in the PCR reactions. The chromatograms were checked, assembled and edited with the CodonCodeAligner 7.0.1 (CodonCode Corporation, Centerville, MA, USA).

DNA extraction was performed using the E.Z.N.A.® Plant DNA Mini Kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's protocol. The internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) were amplified. The PCR reactions were carried out in a thermal cycler with the following settings: denaturation for 4 min at 95 °C, followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 56 °C and extension for 2 min at 72 °C with a final extension at 72 °C for 10 min. The PCR products were purified using an ExoSAP-IT purification kit (Amersham Biosciences, Amersham, UK). The purified samples were sent to the Biological Research Centre, Szeged for sequencing, where amplicons were sequenced using a Sanger Sequencing 3500 Dx Series Genetic Analyzer (Applied Biosystems™, Thermo-Fisher, Waltham, MA, United States). The resulting chromatograms were carefully inspected, assembled, and edited using CodonCode Aligner 7.0.1 (CodonCode Corporation, Centerville, MA, USA). The obtained ITS sequences from the two isolates have been deposited in the GenBank database under the accession numbers OR777263 and OR785012.

For the phylogenetic investigation, a dataset comprising 38 representative *Rigidoporus* sequences was retrieved from the GenBank database. The selection of strains and species for the ITS dataset was based on Oghenekaro et al. (2014), Sell et al. (2014), Wang et al. (2023a), and Wu et al. (2017). The alignment of the ITS data was performed using the online version of MAFFT v. 7, followed by manual correction, trimming, and concatenation using AliView. Subsequently, the dataset was subjected to maximum likelihood (ML) analyses using RaxmlGUI version 7.3.0. The ML analysis yielded the best-scoring tree, which was further refined and edited using MEGA 7 and Adobe Illustrator CS4, respectively. The finalized tree is depicted in Fig. 1.

RESULTS

ITS sequence analyses

The ML phylogenetic analyses were conducted using an ITS dataset consisting of 40 strains from eight *Rigidoporus* species, encompassing 650 characters, including gaps. According to the results, the Hungarian specimens (GenBank nos. OR777263, OR785012) form a well-supported clade (ML = 99%) with other *R. ulmarius* samples collected in England (GenBank nos. AY593868, KJ559446, MZ159373), as well as in two African countries: Cameroon (GenBank no. KJ559445) and Gabon (GenBank no. KU981365). Additionally, samples of "*R. ulmarius*" originating from the American continents (Costa Rica, United States, French Guiana) constitute a closely related yet distinctly separate clade.

Taxonomy

Rigidoporus ulmarius (Sowerby) Imazeki, Bull. Gov. Forest Exp. Stn Tokyo 57: 119 (1952) (Fig. 2)

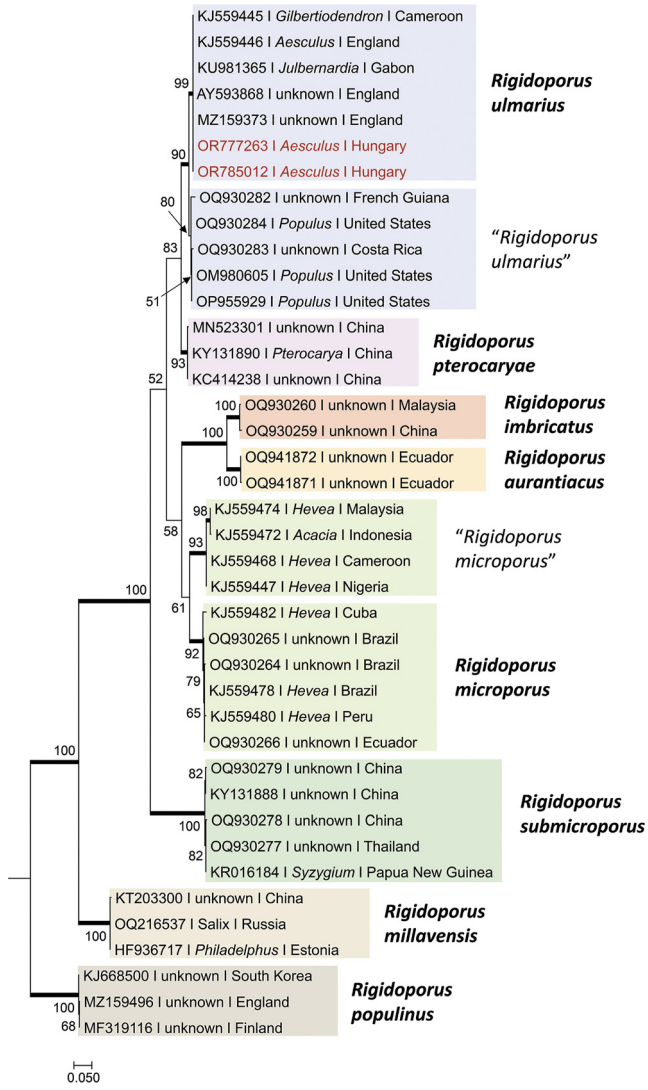

Fig. 1. Phylogeny of *Rigidoporus ulmarius* and related species inferred from RAxML analyses of nrDNA ITS sequences. Topology is from the best scoring Maximum Likelihood (ML) tree. The samples collected in Hungary are marked in red

≡ *Boletus ulmarius* Sowerby, Col. fig. Engl. Fung. Mushr. (London) 1(no. 11): tab. 88 (1797) = *Polyporus cytisinus* Berk., in Smith, Engl. Fl., Fungi (Edn 2) (London) 5(2): 142 (1836). *Fomes cytisinus* (Berk.) Sacc., Syll. fung. (Abellini) 6: 166 (1888)

Description (based on the Hungarian specimens): *Basidiomes* perennial, single or imbricate, often sessile and irregularly shaped, suberous to woody when dry; *sterile surface* smooth or tuberculate, finely tomentose, later glabrous, pale ochraceus to cream color, often with greenish

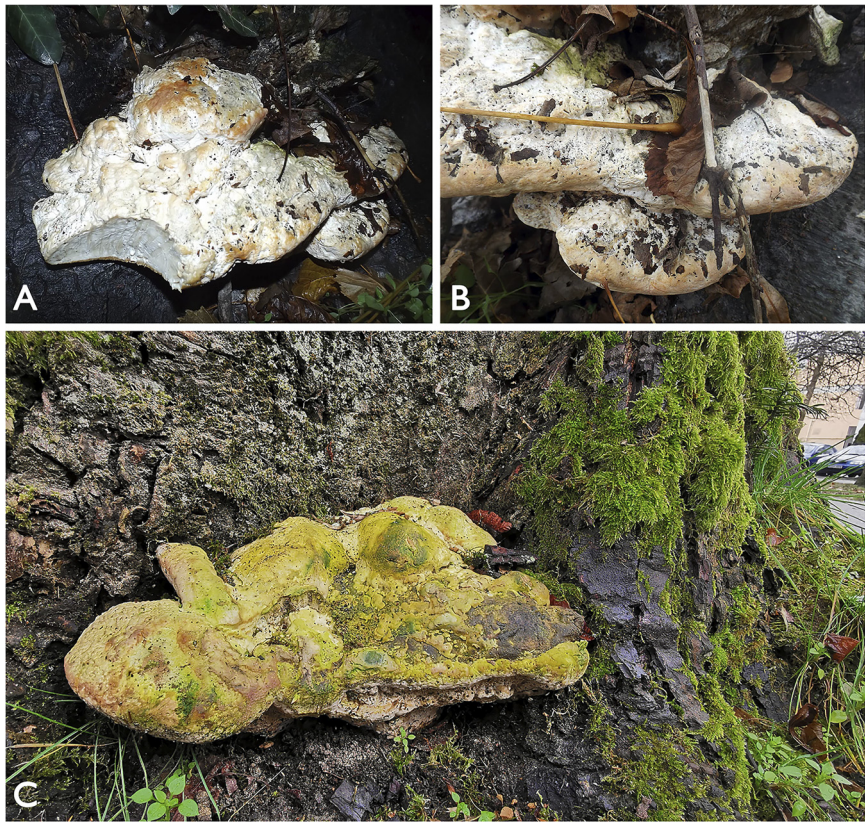

Fig. 2. Basidiomata of *Rigidoporus ulmarius* collected in Hungary. (A–B) basidiomata of *R. ulmarius* collected in Keszthely by J. Vikár. (C) Basidiomata of *R. ulmarius* collected in Nagyatád by L. Vajda. Photos: J. Vikár (A–B), L. Vajda (C)

tints covered by algae; *margin* thick, rounded and pale brown to pale brownish-orange colour; *pore surface* pale brownish-orange when fresh; *pores* round to angular, 5–6 per mm with thin, entire dissepiments; *tube layers* orange, brownish-orange when dry, indistinctly stratified; *context* azonate, whitish firm, corky-fibrous, suberous. *Hyphal system monomitic*; generative hyphae hyaline to pale yellowish, thin-to moderately thick-walled, with simple septa, 3–5 µm in diameter; *cystidia* absent; fusoid, *hymenial cytidioles* present, 11–19 × 5–7 µm, simple-septate at the base. *Basidia* hyaline, clavate, with 4-sterigmata and a simple basal septum, 16–20 × 8–10 µm. *Basidiospores* hyaline, smooth, globose to subglobose, thick-walled, IKI–, (6.5–)6.6–7.2(7.4) × (5.8–)5.9–6.2(–6.4) µm, Qav = 1.1, L = 6.8, W = 6.0, n = (30).

Specimens examined: GREECE. Attica region, Athens, 05.06.2012, on living *Ulmus campestris*, leg. V. Papp, VP-050612-1. HUNGARY. Zala County, Keszthely, 30.11.2017, on living *Aesculus hippocastanum*, leg. J. Vikár, VPapp-301117-VJ, (GenBank no. OR785012). Somogy County, Nagyatád, 10.02.2021, on living *A. hippocastanum*, leg. L. Vajda, VPapp-100221-VL,

(GenBank no. OR777263). IRELAND. County Cork, Castlemartyr, 23.09.2018, on living *A. hippocastaum*, leg. L. Kaposvári, VPapp-230918-KL.

DISCUSSION

After conducting morphological and phylogenetic analyses of samples originating from Hungary, the identity with the species *R. ulmarius* was confirmed. Based on a thorough review of the literature, no previously published data existed for this species in Hungary. Although Igmándy (1970, 1991) did report Hungarian records under the names *Fomitopsis cytisina* (Berk.) Bondartsev & Singer and "*Perenniporia cytisina*", it is evident from his work that these refer not to *R. ulmarius*, but to the species *Vanderbylia fraxinea* (Bull.) D.A. Reid. The latter is a macroscopically somewhat similar necrotrophic polypore species that also causes wood decay at the base of older trees. In Hungary, *V. fraxinea* is not a rare species and is known to affect deciduous tree species from various genera (e.g., *Aesculus*, *Gleditsia*, *Platanus*, *Populus*, *Robinia*, *Quercus*) (Igmándy, 1991; Papp et al., 2011). Due to the potential confusion between these two species, a re-examination of previously collected herbarium specimens identified as *V. fraxinea* might also be necessary. Furthermore, in the future, additional observations and investigations are required for a more comprehensive understanding of the diversity and host-plant preference of *R. ulmarius* in Hungary.

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