**AURANTIPORUS CROCEUS, A FLAGSHIP SPECIES OF THE EUROPEAN FUNGAL CONSERVATION IS RE-DISCOVERED AFTER HALF CENTURY IN HUNGARY**

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**Abstract:** The threatened polypore, *Aurantiporus croceus* has previously been known only from one locality in Hungary, and the basidiome of this species has not been seen in the country since 1972. In this study, a new Hungarian finding of *A. croceus* is reported from an old-growth forest reserve in the Vértes Mts (Central Transdanubia). We present the nrDNA ITS sequence, macro- and microscopical characteristics as well as photographs of the new specimen.

**Keywords:** Central Europe, *Hapalopilus*, Meruliaceae, phylogeny, Polyporales, *Quercus*

**INTRODUCTION**

The peculiar polypore species, *Aurantiporus croceus* (Pers.) Murrill (≡ *Hapalopilus croceus* (Pers.) Bondartsev & Singer) is easily recognised in the field by the often large sized, bright orange-red coloured basidiocarps growing predominantly on veteran oak (*Quercus*) trees. *Aurantiporus croceus* is widely distributed in Europe (Ryvarden and Melo 2014) and also reported from the eastern parts of Asia (Dai 2012) and eastern North America (Zhou *et al.* 2016). Despite its widespread distribution and easily recognisable basidiome, *A. croceus* is considered to be a rare species everywhere. As a result of its rarity and its spectacular appearance, it was placed in the spotlight of the European fungal conservation; it is listed amongst the 33 threatened fungi proposed to be included in the Bern Convention (Dahlberg and Croneborg 2003), and red-listed in 12 European countries (listed as CR in eight countries), and included in nine regional Red Data Books of Russia (Dahlberg 2019). Due to the significantly declined...
population, it became very rare and scattered, it has recently been assessed to the IUCN’s Red List (Dahlberg 2019).

From Hungary, only historical specimens of *A. croceus* were known. These are originated from an old living *Quercus* tree located near Sitke, Western Transdanubia (Igmándy 1968, Szabó 2012). Despite the single locality of this rare polypore, it was not included in the proposed Hungarian Red List (Rimóczi *et al.* 1999). Considering the specific habitat preference and the rarity of *A. croceus* in Hungary, the second author urged to protect this species by law, which was achieved in 2013 (MK 2013). However, despite the greater attention, no new occurrence of this species was observed until 2018. During a mycological survey of Juhdöglő-völgy Forest Reserve (Vértes Mts, Central Transdanubia) in late May, a new location of *A. croceus* was found. In this study the ITS sequence, macro- and microscopical characteristics and photographs of the new Hungarian specimen of *A. croceus* are presented.

**MATERIALS AND METHODS**

**Isolates and morphology**
The new Hungarian specimen was deposited in the private herbarium of the authors. We report the macromorphological descriptions based on field notes. Micromorphological data were obtained from the 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. The following abbreviations were used in the description of the basidiospores: IKI = Melzer’s reagent, IKI− = both inamyloid and indexinoid, 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 (White *et al.* 1990, Gardes and Bruns 1993) were used to amplify the ITS (internal transcribed spacer) region of the nuclear ribosomal DNA. For the amplification we used the
Phire® Plant Direct PCR Kit (Thermo Scientific, USA) following the manufacturer’s recommendations. The PCR (polymerase chain reaction) protocols were set according to Papp and Dima (2017). The quality of PCR products were 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).

The newly generated *Aurantiporus croceus* sequence is deposited in GenBank (Benson et al. 2017); the accession numbers are included in Table 1. For the phylogenetic analysis, similar sequences were searched from GenBank using the BLASTn search tool (Altschul et al. 1990). The ITS region was aligned with PRANK (Löytynoja and Goldman 2005, 2008) as implemented in its graphical interface (PRANKSTER) using default settings. SeaView 4 (Gouy et al. 2010) was used to visually inspect and improve the alignment. The nucleotide dataset resulted an alignment length of 697 characters. The dataset was subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses, which were performed in raxmlGUI (Silvestro and Michalak 2012) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. ML analysis was done using 1000 rapid bootstrap searches. For the nucleotide partition the GTRGAMMA substitution model, while for the indel partition the RAxML default set for binary characters were applied. In the BI analysis the GTR + G model of evolution for the nucleotide partition, and the two-parameter Markov model (Mk2 Lewis) for the indel partition were applied. The BI settings were: four Markov chain Monte Carlo (MCMC) over 5 million generations, sampling every 1000th generation, two independent runs, and burn-in of 20% (the first 1000 trees were discarded). Post burn-in trees were used to compute a 50% majority rule consensus phylogram. Phylogenetic trees from both ML and BI analyses resulted in congruent topologies. The best scoring tree from the ML analysis was edited with MEGA6 (Tamura et al. 2013) and presented in Figure 1.
**Table 1.** Details of the specimens comprised in this study. Species, herbarium voucher numbers, country, and GenBank accession numbers are presented.

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen voucher</th>
<th>Collection site</th>
<th>GenBank number</th>
<th>References</th>
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<td><em>A. croceus</em></td>
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<td><strong>Hungary</strong></td>
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<td><strong>this study</strong></td>
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<td>MIETTINEN et al. (2016)</td>
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</table>

¹ as *Hapalopilus*; ² as *Aurantiporus*; ³ as *Tyromyces*
Figure 1. Phylogenetic tree of *Aurantiporus croceus* and related poroid species in Meruliaceae inferred from MrBayes and RAxML analyses of the nrDNA ITS sequences based on the best scoring maximum likelihood (ML) tree. *Hapalopilus rutilans* served for outgroup. Bayesian posterior probabilities (PP) > 0.9 and ML bootstrap values > 50% as evidences of statistical support are shown above or below branches. The bar indicates 0.1 expected change per site per branch.

RESULTS

**ITS sequence analyses**
The dataset represents 21 sequences of eight poroid Meruliaceae species, with *Hapalopilus rutilans* as outgroup. The Hungarian specimen (GenBank: MT876120) cluster together with other *A. croceus* specimens (labelled as *Hapalopilus croceus*) collected in
Czech Republic (GenBank: JQ821320) and Lithuania (GenBank: MH571407). The sequences of other morphologically similar European species formerly discussed in *Aurantiporus* (viz. *Odoria alborubescens* (Bourdot & Galzin) V. Papp & Dima and *Pappia fissionalis* (Berk. & M.A. Curtis) Zmitr.) cluster in well-separated strongly supported clades, respectively.

**Taxonomy**


(Figures 2–3)


Basidiocarps annual, occasionally semi-perennial (Figure 3), broadly attached, pileate; upper surface bright orange-yellow, at first, and finely pubescent, becomes vivid orange later, finely brownish orange and almost smooth when old; flesh vivid orange, dark wine-red to almost black when touched with KOH. Context soft and watery when fresh, shrinking considerably and becomes hard and rigid when dry, taste sourish or slightly bitter. Pore surface bright reddish-orange when fresh, brownish when dry, pores angular, 2–3 per mm. Tubes 2–3 cm thick, bright orange, spongy and watery when fresh, drying darker orange to brownish, becomes hard and resinous. Hyphal system monomitic, hyphae hyaline and thin-walled, moderately branched, 3–6 µm in diam., septa with clamps. Hyphae richly encrusted with golden yellow crystals, forming a dense and loose covering around the wall. Yellow incrustation layer lose its elements in small pieces easily. Basidia 4-spored, 18–30 × 7–10 µm, clamped. Cystidia or other sterile elements absent. Basidiospores broadly ellipsoid, (4.02–)4.12–4.36(–4.48) × (2.82–)3.00–3.22(–3.26) µm, L = 4.25 µm, W = 3.1 µm, Q = 1.37 (n = 30), hyaline, thin-walled, smooth, negative in Melzer’s reagent.

**Specimens examined**: HUNGARY. Vas County, near Sitke, Bajti, on old living *Quercus robur*, leg. Z. Igmándy, Pagony et Varga, 14 Sept 1964 (Igmándy 1488); leg. Haracs et Igmándy, 17 Sept 1965
(Igmándy 1599), leg. Z. Igmándy et Varga, 3 Dec 1966 (Igmándy 1675); leg. Z. Igmándy, 8 Nov 1968 (Igmándy 1807); leg. Z. Igmándy, 28 Oct 1969 (Igmándy 1848); leg. Z. Igmándy, 11 Oct 1972 (Igmándy 2010); Fejér County, Vértes Mts, near Csákberény, Juhdöglő-völgy Forest Reserve, on the underside of large *Quercus* sp. log, leg. A. Koszka et V. Papp, 30 May 2018 (VPapp 300518-1), GenBank: MT876120. CZECH REPUBLIC. Moravia, on *Quercus robur*, leg. A. Černý, 2 May 1958 (Igmándy 10129).

Figure 2. Macromorphology and habitat of *Aurantiporus croceus*. a–b: habitat in Juhdöglő-völgy Forest Reserve. c–e: basidiomata. f: KOH reaction of the basidiomata. g: pore surface (fresh material). Photos (a, b: V. Papp, c, e, f, g: A. Koszka, d: P. Finy).
Figure 3. Cross-section of *Aurantiporus croceus* basidiome (dried material). C1: old context, C2: new context, T1: old trama, T2: new trama.

**DISCUSSION**

Most observations of *A. croceus* are originated from living, old and coarse veteran trees, mainly oak (*Quercus*) and more rarely on chestnut (*Castanea*) in parks and old growth forests (Dahlberg 2019). The new Hungarian location of *A. croceus* is reported from an old oak forest site at the Juhdöglő-völgy Forest Reserve, which is considered as one of the few European primary forests extant in...
the Pannonian Biogeographic region (Sabatini et al. 2018). The lignicolous fungi of the Juhdögő-völgy Forest Reserve have intensely been studied in the last decade, and several rare and threatened wood-inhabiting basidiomycetes were documented (Papp 2011, 2012, Papp and Szabó 2013, Papp and Dima 2014, 2018, Papp et al. 2012, 2016; Crous et al. 2018, Liu et al. 2018).

Despite of the consecutive, targeted search of *A. croceus* at the old oak forest sections of the core area, only one location was observed. The basidiocarp grew 10 meters from the root zone of an old oak log (*Figure 2 a,b*). Although Juhdögő-völgy Forest Reserve are protected and no forest management practices are being applied on the collection site, the crowded population of boar, red deer and mouflon creates a discontinuity in the forest. Thus, the genotype (or population) of *A. croceus* investigated in the Juhdögő-völgy Forest Reserve is threatened by the decreasing habitat quality.

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