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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 indextrinoid, 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.

Species	Specimen voucher	Collection site	GenBank number	References
Aurantipileus mayanensis	TJB10228, type	Belize	HM772140	GINNS *et al.* (2010)
A. mayanensis	JV 1504/128	Costa Rica	KT156706	unpublished
Aurantiporus croceus[1]	BRNM 737561	Czech Republic	JQ821320	DVOŘÁK *et al.* (2014)
A. croceus[1]	H6-27	Lithuania	MH571407	unpublished
A. croceus	VPapp 300518-1	**Hungary**	MT876120	**this study**
Geesterania carneola	MCW 474/13	Brazil	KY175000	WESTPHALEN *et al.* (2018)
G. carneola	MCW 388/12	Brazil	KY174999	WESTPHALEN *et al.* (2018)
G. davidii	MCW 396/12, type	Brazil	KY174998	WESTPHALEN *et al.* (2018)
G. davidii	MCW 370/12	Brazil	KY174997	WESTPHALEN *et al.* (2018)
Luteoporia albomarginata	Dai 15229, type	China	KU598873	WU *et al.* (2016)
L. albomarginata	GC 1702-1	Taiwan	MF496147	CHEN *et al.* (2018a)
L. albomarginata	TNM GC 1702-1	Taiwan	LC379003	CHEN *et al.* (2018b)
Odoria alborubescens	PC 0706595, type	France	MG097863	PAPP & DIMA (2018)
O. alborubescens	BP 106943	Hungary	MG097864	PAPP & DIMA (2018)
O. alborubescens[2]	BRNU 627479	Czech Republic	JQ821319	DVOŘÁK *et al.* (2014)
Pappia fissilis[3]	SFC20140626-03	South Korea	KX792915	KIM *et al.* (2016)
P. fissilis[3]	BRNM 699803	Czech Republic	HQ728292	TOMŠOVSKÝ (2012)
P. fissilis[3]	HHB9530sp	USA	KY948774	JUSTO *et al.* (2017)
Phlebiporia bubalina	Dai 13168, type	China	KC782526	CHEN & CUI (2014)
P. bubalina	Dai 15231	China	KU598876	WU *et al.* (2016)
P. bubalina	Dai 9798	China	KY131842	WU *et al.* (2017)
Hapalopilus rutilans	JV0407/34J	USA	KX752619	MIETTINEN *et al.* (2016)
H. rutilans	H 6012735	Finland	KX752614	MIETTINEN *et al.* (2016)

[1] as *Hapalopilus*; [2] as *Aurantiporus*; [3] as *Tyromyces*

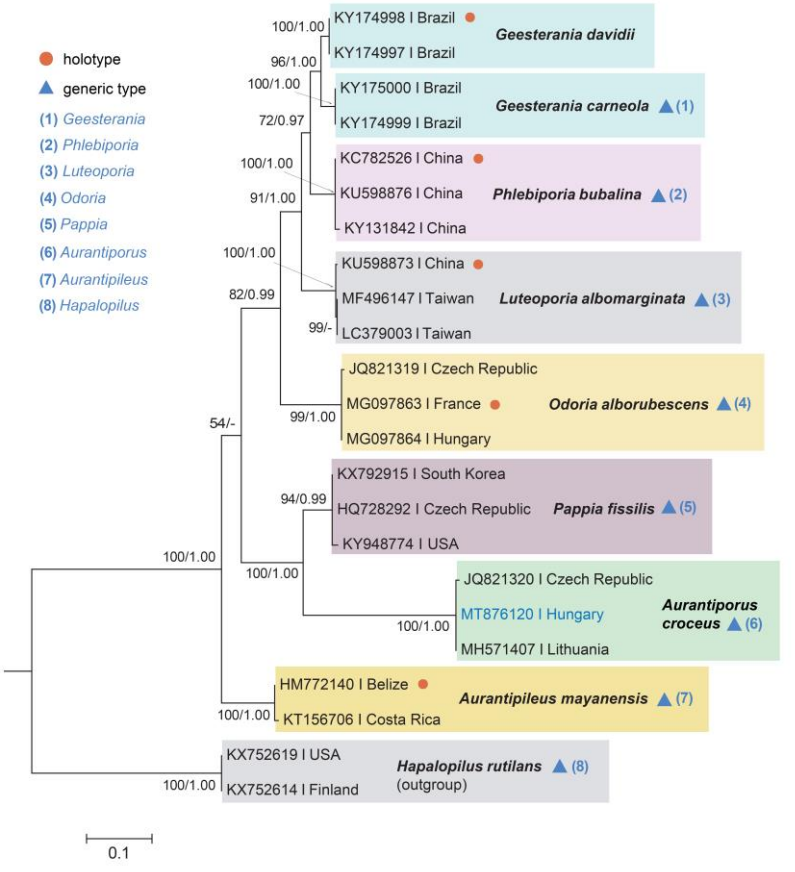

Figure 1. Phylogenetic tree of *Aurantiporus croceus* and related poroid species in Meruliaceae inferred from MrBayes and RAxML analyses of the nrDNA ITS sequences based on the best scoring maximum likelihood (ML) tree. *Hapalopilus rutilans* served for outgroup. Bayesian posterior probabilities (PP) > 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 fissilis* (Berk. & M.A. Curtis) Zmitr.) cluster in well-separated strongly supported clades, respectively.

Taxonomy

Aurantiporus croceus (Pers.) Murrill, Mycologia 12(1): 11, 1920 (*Figures 2–3*)

≡ *Boletus croceus* Pers., Observationes mycologicae 1: 87, 1796; ≡ *Polyporus croceus* (Pers.) Fr., Observ. mycol. (Havniae) 1: 124, 1815; ≡ *Phaeolus croceus* (Pers.) Pat., Essai taxonomique sur les familles et les genres des Hyménomycètes: 86, 1900; ≡ *Hapalopilus croceus* (Pers.) Bondartsev & Singer, Annales Mycologici 39 (1): 52, 1941; ≡ *Tyromyces croceus* (Pers.) J. Lowe, Mycotaxon 2 (1): 21, 1975

= *Polyporus pilotae* Schwein., Transactions of the American Philosophical Society 4 (2): 156, 1832

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, spongiose 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. Haracsi 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).

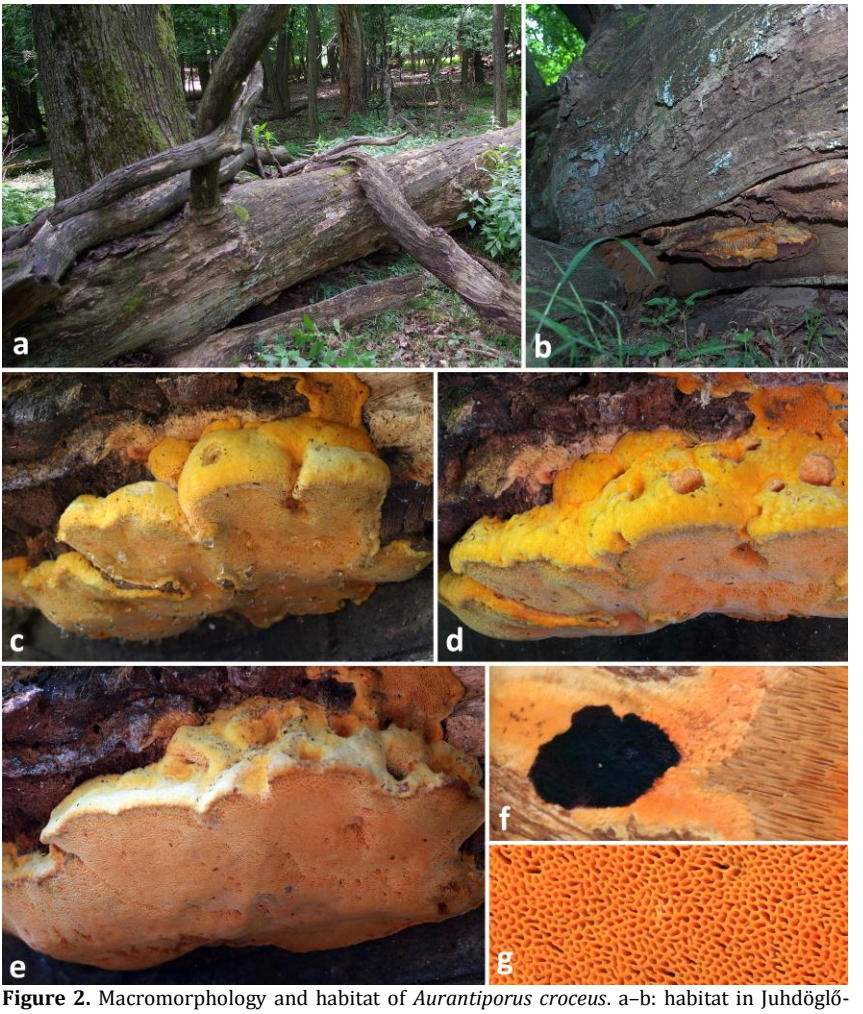

Figure 2. Macromorphology and habitat of *Aurantiporus croceus*. a–b: habitat in Juhdöglő-völgy Forest Reserve. c–e: basidiomata. f: KOH reaction of the basidiomata. g: pore surface (fresh material). Photos (a, b: V. Papp, c, e, f, g: A. Koszka, d: P. Finy).

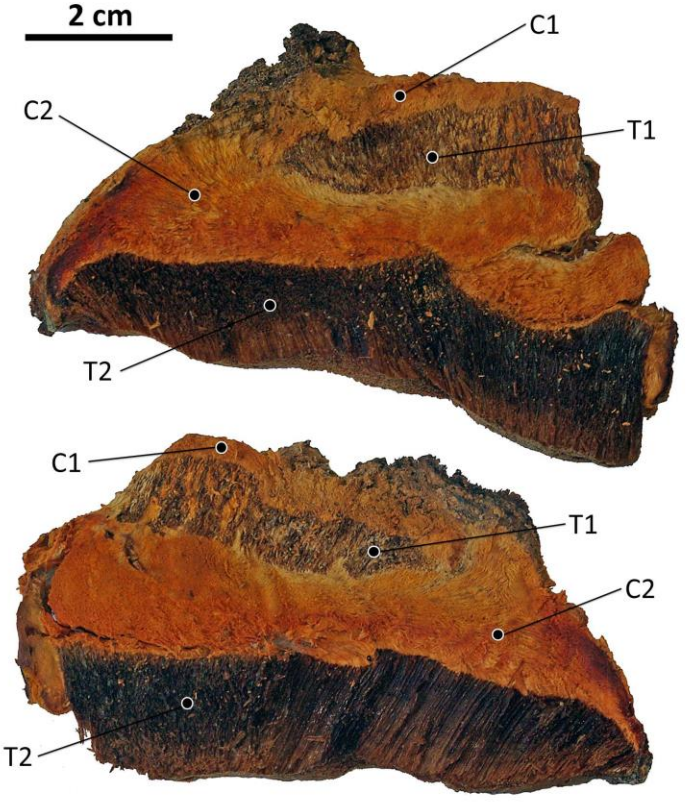

Figure 3. Cross-section of *Aurantiporus croceus* basidiome (dried material). C1: old context, C2: new context, T1: old trama, T2: new trama.

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 funga of the Juhdöglő-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 Juhdöglő-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 Juhdöglő-völgy Forest Reserve is threatened by the decreasing habitat quality.

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