

## ***Rigidoporus pouzarii*, a new polypore species related to *Rigidoporus crocatus***

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The new polypore *Rigidoporus pouzarii* Vampola et Vlasák is described, occurring in alluvial forests, forming annual resupinate fruitbodies on lying dead stems of hardwoods, mostly *Alnus* species. In Europe, the species has been probably misidentified as *R. crocatus*, a closely related species differing by perennial, multi-layered fruitbodies and occurrence in old-growth mountain forests, preferentially on logs of *Abies alba*. Diagnostic features of both species are discussed and DNA sequences are also added. The name is dedicated to Zdeněk Pouzar, distinguished Czech mycologist, expert in alluvial forest fungi and our excellent teacher, on the occasion of his 80th birthday.

**Key words:** *Rigidoporus*, taxonomy, internal transcribed spacer, alluvial forest fungi.

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Je popsán nový druh chorošů *Rigidoporus pouzarii* Vampola et Vlasák, který roste v lužních lesích a tvoří jednoleté rozlité plodnice na ležících kmenech výhradně listnáčů, nejčastěji olší. V Evropě byl tento druh dosud zaměňován s příbuzným *R. crocatus*, který se však liší víceletými vrstevnatými plodnicemi a růstem převážně na ležících kmenech *Abies alba* v horských pralesích. Hlavní rozdílné diagnostické znaky obou druhů jsou diskutovány a je připojena molekulární charakteristika. Nový druh je věnován Zdeňku Pouzarovi, významnému českému mykologovi, nadšenému znalci hub lužních lesů a našemu obětavému učiteli, k jeho 80. narozeninám.

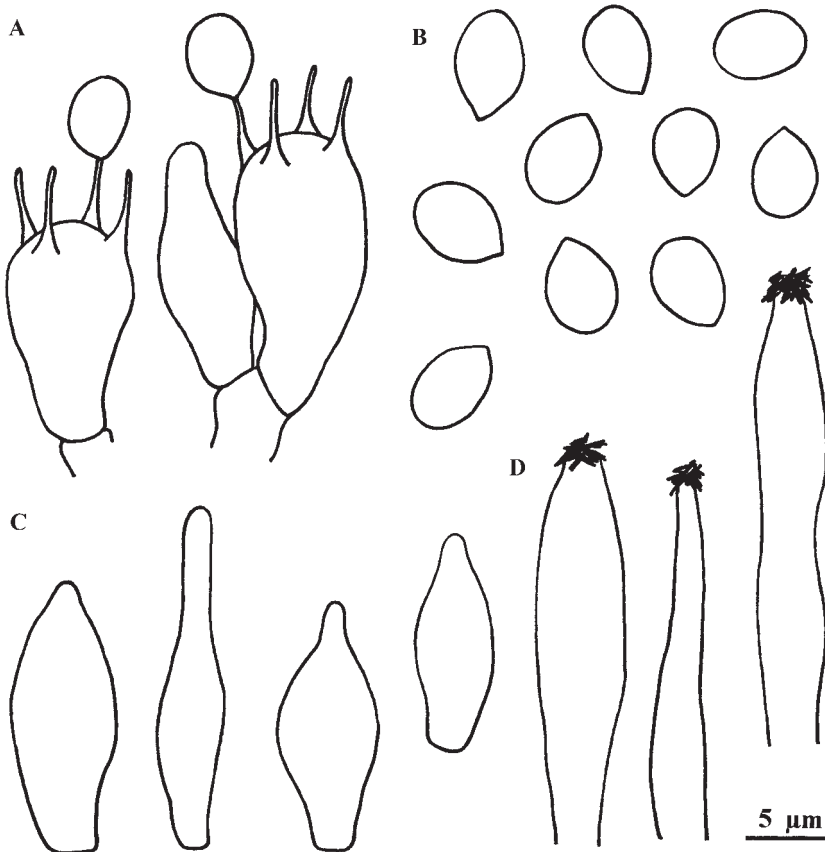
### INTRODUCTION

Since the beginning of the 1990s, our survey of polyporoid fungi in alluvial forest nature reserves Šúr near Bratislava (Slovakia) and Raňšpurk near Lanžhot (Czech Republic) have yielded repeatedly a strange *Rigidoporus* species which was at first considered a lowland, annual form of *R. crocatus* (Pat.) Ryvarden [syn. *R. nigrescens* (Bres.) Donk]. In contrast to the perennial, distinctly multi-layered and “café-au-lait” coloured fruitbodies of *R. crocatus*, this fungus always creates annual fruitbodies which are at first whitish or light yellow-pinkish (Fig. 3)

showing striking red spots after bruising (Fig. 4) and only later acquire the typical “café-au-lait” colour (Fig. 5). Molecular analysis confirmed that it is a different species which is described here as new to science.

#### MATERIALS AND METHODS

**Macroscopic and microscopic study.** Macroscopical and microscopic characters were studied on fresh fruitbodies and herbarium specimens deposited in the herbaria PRM (National Museum Prague, Mycological Department, Czech Republic), MJ (Muzeum Vysočiny, Jihlava, Czech Republic), CWU



**Fig. 1.** *Rigidoporus pouzarii* (PRM 899856, holotype). A – basidia, B – spores, C – fusoid cystidioles, D – hyphoid cystidia. Del. P. Vampola.

(V.N. Karasin National University, Kharkiv, Ukraine), and in the private herbaria of both authors. Microscopic characteristics were observed in Melzer's reagent and Cotton Blue under Olympus BX41 and Meopta D816Bi microscopes with an oil immersion lens at a magnification of 1000×. A total of 20 spores from each specimen were measured.

**DNA isolation and sequencing.** The fungal tissue (0.25 g) was disintegrated for 60 s with an MM301 (Retch) steel ball mixer mill at room temperature. DNA was isolated using the CTAB/NaCl extraction buffer as described by Murray and Thompson (1980), followed by repeated extraction with chloroform and isopropanol precipitation. Crude DNA was dissolved in 100 µl of sterile water and further purified using the Wizard Clean Up kit (Promega). The resulting DNA solution (50 µl) was diluted ten times and 1 µl was used as a template for PCR amplification with the ITS5 and ITS4 primers (White et al. 1990) in 25 µl reaction mixture using 55 °C annealing temperature. Amplified DNA was sequenced in the Genomics laboratory of the Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on an ABI 3730xl DNA analyzer, using the BigDye Terminator 3.1 kit.

**Phylogenetic analysis.** The sequences were aligned with Clustal X and manually pruned. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). Initial tree(s) for the heuristic search were obtained using the BIONJ method with an MCL distance matrix. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The final dataset contained a total of 573 positions, which included 485 constant and 88 variable sites. Phylogenetic analyses were conducted in MEGA5 (Tamura et al. 2011).

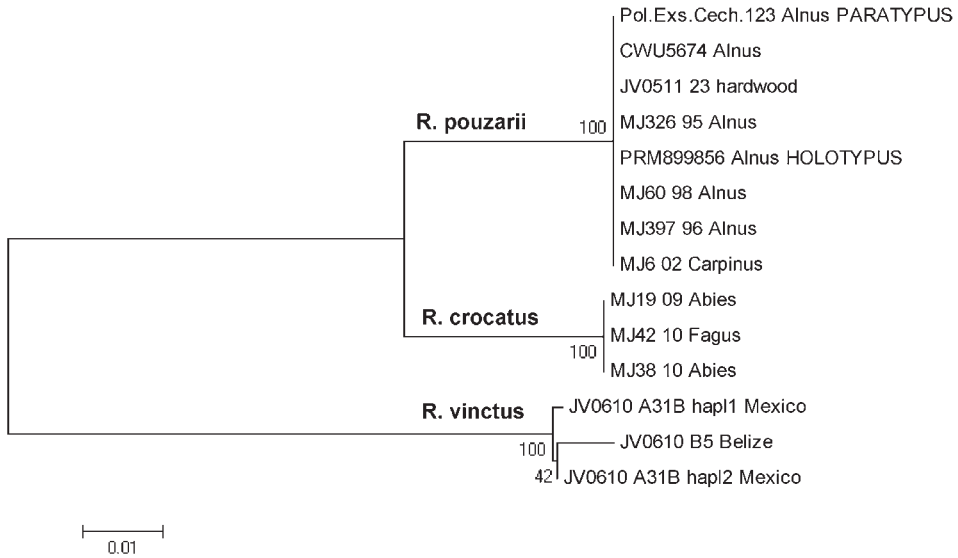
## RESULTS AND DISCUSSION

### ***Rigidoporus pouzarii* Vampola et Vlasák, spec. nov.**

(Mycobank MB 564573)

**Diagnosis latina.** Carposomata annua, resupinata, primo albidula, dein pallide rosea; pori angulato-rotundati, parvi, cca 5–7 per 1 mm; systema hypharum monomiticum, cum hyphis generativis hyalinis, tenuiter tunicatis usque crasse tunicatis, efibulatis; hymenium e basidiis, cystidiolis et cystidiis constat; hyphoidea cystidia tenuiter tunicata, hyalina, apicibus incrustati; basidiosporae 4,7–6,3 × 4–4,6 µm, globoso-ovoideae vel breviter ellipsoideae, hyalinae. Ad ligna arborum frondosarum. Habitu macroscopico *Rigidoporo crocato* similis, sed carposomata annua.

**Holotypus.** Slovacia, Svätý Jur, area tuta "Šúr", 15 km sept.-or. versus Bratislava, alt. 130 m s. m., *Alnus glutinosa* – ad truncum iacentem, 19.VI. 2004 leg. P. Vampola no. 2704 [holotypus in herbario Musei Nationalis Pragae (PRM 899856) asservatur (GenBank JQ409462)]; isotypus in herbario Musei Iglaviensis



**Fig. 2.** Evolutionary relationships of 13 *Rigidoporus* specimens based on a sequence comprising the ITS1, 5.8S and ITS2 regions of the ribosomal RNA gene. The evolutionary history was inferred using the Maximum Likelihood method; the tree with the highest log likelihood (1205.0545) is shown. Bootstrap support of tree topology is shown next to the branches. The tree is drawn to scale, with branch lengths relative to the number of substitutions per site. Accession numbers of studied vouchers are given in “Specimens examined”. In *R. vincetus* specimen JV0610/A31B, two haplotypes differing by short insertions/deletions were found.

(MJ 27/04); *paratypi* in Vampola (1995), Polyporales Exs. Čechosl. no. 123 (ut *Rigidoporus nigrescens*), GenBank JQ409464.

**Etymology.** Named in honour of Zdeněk Pouzar, eminent Czech mycologist.

**Description.** Fruitbodies annual, resupinate, 2–4 mm thick, covering the substrate for up to dozens of cm<sup>2</sup>, white or cream and somewhat translucent when fresh, later yellowish, pale pinkish to pale buff, drying pinkish brown. Subiculum membranaceous, tubes thin-walled, up to 3 mm long. Pores circular to angular, about 5–7 per mm, in fresh fruitbodies staining bright red 2 to 3 minutes after bruising (Fig. 4), drying sometimes smoky grey on pore edges (Fig. 6). Hyphal system monomitic, tramal hyphae thin-walled to somewhat thick-walled, hyaline, simple-septate, 3–4.5 µm in diam., subicular hyphae similar, 3–8 µm in diam. Basidia broadly clavate, 4-sterigmate, 15–22 × 7–10 µm, with basal simple septum. Fusoid or lageniform cystidioles present, 12–22 × 5–7 µm. Hyphoid, thin-walled cystidia 3–5 µm in diam., often with apical incrustation, are abundant close to the pore edges. Spores broadly ellipsoid to ovoid, hyaline, thin-walled, 4.7–6.3 × 4.0–4.6 µm (Fig. 1).

**Ecology.** On fallen dead trunks of hardwoods (*Acer campestre*, *Alnus glutinosa*, *Carpinus betulus*, *Ulmus laevis*) in lowland forests of warmer regions, at altitudes up to 150 m. All existing records are from nature reserves of alluvial old-growth forests with a long history of vegetation continuity. In the Czech Republic, the only known habitat can be characterised as hardwood forest of lowland rivers (altitude 150 m); habitats in Slovakia and Ukraine as alder carrs (altitude 130 and 100 m). Although undoubtedly a very rare species, *Rigidoporus pouzarii* occurs at both Czech and Slovak localities each year on about ten fallen trunks in all stages of decay, causing a white rot. Fruitbodies grow from spring to autumn, exceptionally also in mild winters, and are annual, with only one layer of tubes. Quite seldom, in early spring, fragments of withered tubes of the preceding year can be seen under newly developed fresh fruitbodies.

**Distribution.** Currently known from the Czech Republic, Slovakia and Ukraine. It probably occurs also in alluvial forests in other countries and not only in Europe, but it is erroneously being identified as *R. crocatus* (= *R. nigrescens*).

**Notes.** *Rigidoporus pouzarii* is undoubtedly closely related to *R. crocatus*, differing macroscopically by annual, at first whitish or pale pinkish fruitbodies which show a striking red staining after bruising. Although we could not study the type of *R. crocatus* collected in Tunis on *Quercus mirbeckii* (Patouillard 1894), we accept its presumed identity with *Poria nigrescens* Bres., as published by Ryvar den (1983). In his original description, Patouillard did not mention the multi-layer character of its fruitbodies, but he described the tubes to be up to 10 mm long in the centre and much shorter towards the margin. This is also typical of *P. nigrescens* fruitbodies. The photos of typical multi-layered *P. nigrescens* were published on the Internet (Vlasák 2011). Bresadola described *P. nigrescens* using a collection from *Abies alba*, Mt. Sitno, Slovakia, a locality well known to us. Similarly as in other mountain old-growth forests of the Czech and Slovak Republics, *Rigidoporus crocatus* (= *R. nigrescens*) grows here making multi-layered fruitbodies mostly on fallen logs of *Abies alba* and only rarely on hardwoods. *Rigidoporus pouzarii* prefers warmer regions, where it grows on lying hardwood logs, most often alders (especially *Alnus glutinosa*). A very similar species is also *Poria endoxantha* Petch described in 1922 from Ceylon and known also from Florida, USA (Lowe 1966). This species has, however, thick-walled cystidia. The cystidia are always thin-walled in *Rigidoporus pouzarii*. According to Corner (1987) *Poria endoxantha* is identical with *Rigidoporus albostygius* (Berk. et M.A. Curtis) Rajchenb., a taxon now placed in the synonymy of *R. vinctus* (Berk.) Ryvar den. In our opinion, the identity of *Poria endoxantha* should be re-evaluated using modern molecular methods. After many years of studying abundant fresh and herbarium material we conclude that the mycological literature contains many inaccurate data and mistakes in *Rigidoporus* species descriptions, and many misinterpretations of species, making the identification of some collec-

tions very difficult. Also from Asia (Cui et al. 2009, Dai 1998, Núñez & Ryvarden 1999, Núñez et al. 2001) several new species with confusingly similar features were recently described, which we have not been able to study so far. Accordingly, we are preparing a more extensive and more fundamental study of the *Rigidoporus* / *Physisporinus* species complex to be published later.

### ITS sequence analysis

The ITS rRNA region of eight *Rigidoporus pouzarii* specimens from three known localities were sequenced together with three specimens of *R. crocatus* from *Abies alba* and *Fagus sylvatica* (Fig. 2). The tropical *Rigidoporus vinctus* (Berk.) Ryvarden with similar basidiomes, hyphae and spores but no colour change and with thick-walled cystidia was also included in the study to root the tree.

The ITS1 and ITS2 regions of *R. pouzarii* and *R. crocatus* show the same length but the sequence variation is considerable. A 10% (19/200) difference in the ITS1 and 5% (10/196) in the ITS2 region between *R. pouzarii* and *R. crocatus* was detected. This suggests two well-separated species. Among different *R. pouzarii* specimens, the sequence is identical except for five heterozygous sites.

### Specimens examined

*Rigidoporus crocatus*. Czech Republic. Novohradské hory Mts.: Žofín (distr. Český Krumlov), Žofínský prales Nature Reserve, alt. 760 m, on fallen trunk of *Abies alba*, 19. IX. 2010 leg. et det. P. Vampola (MJ 38/10, GenBank JQ409467); *ibid.*, on fallen trunk of *Fagus sylvatica* (MJ 42/10). Slovakia. Veporské vrchy Mts.: Čierny Balog (distr. Brezno), Dobročský prales Nature Reserve, alt. 900 m, on fallen trunk of *Abies alba*, 26. IX. 2009 leg. et det. P. Vampola (MJ 19/09, GenBank JQ409466); Kremnické vrchy Mts.: Badín (distr. Banská Bystrica), Badínský prales Nature Reserve, alt. 700 m, on fallen trunk of *Abies alba*, 28. IX. 2009 leg. et det. P. Vampola (MJ 33/09).

*Rigidoporus pouzarii*. Czech Republic. Lanžhot (distr. Břeclav), Ranšpurk Nature Reserve, alt. 150 m, on fallen trunk of *Ulmus laevis*, 3. VI. 1964 leg. F. Kotlaba et Z. Pouzar, VII. 1971 det. Z. Pouzar as *Rigidoporus nigrescens* (PRM 627557), *ibid.*, on fallen trunk of *Alnus glutinosa*, 20. XI. 1996 leg. et det. P. Vampola (MJ 397/96); *ibid.*, 22. X. 1998 leg. et det. P. Vampola (MJ 60/98); *ibid.*, on fallen trunk of *Acer campestre*, 20. X. 2001 leg. et det. P. Vampola (MJ 27/01); *ibid.*, on fallen trunk of *Carpinus betulus*, 2. III. 2002 leg. et det. P. Vampola (MJ 6/02, 7/02); *ibid.*, on fallen hardwood trunk, 19. XI. 2005 leg. et det. J. Vlasák (JV0511/23, GenBank JQ409465). Slovakia. Svätý Jur (distr. Bratislava), Šúr Nature Reserve, alt. 130 m, on fallen trunk of *Alnus glutinosa*, 19. VI. 2004 leg. P. Vampola (holotypus: PRM 899856, GenBank JQ409462), *ibid.*, 12. X. 1993 leg. et det. P. Vampola as *Rigidoporus nigrescens* (paratypus: Pol. Exs. Čech. 123, MJ 600/93-3326, GenBank JQ409464), *ibid.*, 12. X. 1995 leg. et det. P. Vampola (MJ 326/95, GenBank JQ409463). Ukraine. Kharkiv Region, Balaklea District, Syverskyi Donets River floodplain, Iziumska luka Regional Landscape Park, alt. 100 m, on fallen trunk of *Alnus glutinosa*, 6. XI. 2010 leg. A.Yu. Akulov and A.V. Ordynets, 4. I. 2012 det. A.V. Ordynets as *Rigidoporus crocatus* (CWU/Myc/5674, GenBank JQ733558).

*Rigidoporus vinctus*. Mexico. Los Tuxtlas Reserve, hardwood, 12. X. 2006 leg. J. Kout, det. J. Vlasák (JV0610/A31B, GenBank JQ409460, JQ409461). Belize. Cockscomb Basin, hardwood, 27. X. 2006 leg. J. Kout, det. J. Vlasák (JV0610/B5, GenBank JQ409459).





**Fig. 3.** *Rigidoporus pouzarii* (PRM 899856, holotype), juvenile fruitbody. Slovakia: Svätý Jur near Bratislava, Šúr Nature Reserve, *Alnus glutinosa*, 19. VI. 2004. Photo: P. Vampola.



**Fig. 4.** *Rigidoporus pouzarii* (PRM 899856, holotype), juvenile fruitbody when bruised. Slovakia: Svätý Jur near Bratislava, Šúr Nature Reserve, *Alnus glutinosa*, 19. VI. 2004. Photo: P. Vampola.



**Fig. 5.** *Rigidoporus pouzarii* (JV0511/23), mature fruitbody. Czech Republic: Lanžhot (distr. Břeclav), Ranšpurk Nature Reserve, on fallen hardwood trunk, 19. XI. 2005. Photo: J. Vlasák.



**Fig. 6.** *Rigidoporus pouzarii* (MJ 600/93-3326, paratype), fragment of dry fruitbody. Slovakia: Svätý Jur near Bratislava, Šúr Nature Reserve, *Alnus glutinosa*, 12. IX. 1993. Photo: P. Vampola.



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