

***Aurantiporus alborubescens* (Basidiomycota, Polyporales)  
– first record in the Carpathians  
and notes on its systematic position**

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Dvořák D., Běťák J., Tomšovský M. (2014): *Aurantiporus alborubescens* (Basidiomycota, Polyporales) – first record in the Carpathians and notes on its systematic position. – Czech Mycol. 66(1): 71–84.

The authors present the first collection of the rare old-growth forest polypore *Aurantiporus alborubescens* in the Carpathians, supported by a description of macro- and microscopic features. Its European distribution and ecological demands are discussed. LSU rDNA sequences of the collected material were also analysed and compared with those of *A. fissilis* and *A. croceus* as well as some other polyporoid and corticioid species, in order to resolve the phylogenetic placement of the studied species. Based on the results of the molecular analysis, the homogeneity of the genus *Aurantiporus* Murrill in the sense of Jahn is questioned.

**Key words:** *Aurantiporus*, phylogeny, old-growth forests, beech forests, indicator species.

Dvořák D., Běťák J., Tomšovský M. (2014): *Aurantiporus alborubescens* (Basidiomycota, Polyporales) – první nález v Karpatech a poznámky k jeho systematickému zařazení. – Czech Mycol. 66(1): 71–84.

Autoři prezentují první nález vzácného choroše přirozených lesů, druhu *Aurantiporus alborubescens*, v Karpatech, doprovázený makroskopickým i mikroskopickým popisem. Je diskutováno rozšíření druhu v Evropě a jeho ekologické nároky. Pro zjištění fylogenetické pozice studovaného druhu byly analyzovány sekvence LSU rDNA z nalezených plodnic a porovnány se sekvencemi z druhů *A. fissilis*, *A. croceus* a některých dalších taxonů. Na základě výsledků molekulární analýzy je zpochybněna homogenita rodu *Aurantiporus* Murrill ve smyslu Jahna.

## INTRODUCTION

In late June 2011, during a mycological survey of Holý kopec Nature Reserve in the Chřiby hills in SE Moravia, an interesting polypore species reminding *Aurantiporus fissilis* (Berk. & M.A. Curtis) H. Jahn was collected on a fallen beech trunk by the second author. Fruitbodies were characterised by an orange to pinkish

brick, pubescent-strigose pileus surface and strong sweet smell, and tentatively assigned to *Aurantiporus alborubescens* (Bourdot & Galzin) H. Jahn by the first author. Subsequent study of microscopic characters and comparison with literature confirmed this identification. Since the species had not been reported from the Carpathians and adjacent regions of Central Europe before and is very rare throughout its distribution range, we describe it here, adding some insights into its taxonomy and ecology. Also, due to insufficient information on the systematic position of this species, preliminary data based on a molecular analysis are presented.

#### MATERIAL AND METHODS

The macroscopic description is based on a study of fresh specimens. Microscopic slides were mounted from dried material in Melzer's solution, 10% ammoniacal Congo Red and 5% aqueous solution of KOH, using an Olympus BX41 light microscope. The size of mature spores was measured on 50 spores using the Cell-B software (© Olympus Digital Image Systems) in Melzer's solution.  $Q$  represents the length-width ratio of individual spores,  $Q_{av}$  is the average of these measurements.

Abbreviations of public herbaria follow Thiers (on-line).

**DNA extraction and amplification.** Herbarium specimen BRNU 627479 of *A. alborubescens* was used for DNA analyses. In addition, a specimen of *A. croceus* (BRNM 737561) was co-analysed to find out evolutionary relations between these two species. DNA was isolated from dried fungal material using the DNeasy Plant Mini kit (Qiagen, Germany).

DNA fragments encompassing the ITS and partial nLSU (including D1/D2 domains) of rRNA were amplified using the following primer combinations: ITS1/ITS4, ITS5/ITS4 Basidiomycete, or LR0R (ViU)/LR6 (Lutzoni on-line, Nikolcheva & Bärlocher 2004, White et al. 1990).

The DNA was amplified with PCR as in earlier studies (Tomšovský et al. 2010, Tomšovský 2012), using a Mastercycler\_ep thermocycler (Eppendorf, Germany). Amplicons were custom-purified and sequenced at Macrogen, Seoul (Republic of Korea). The sequences were deposited in the NCBI Nucleotide Sequence Database.

**Phylogenetic analysis.** ITS data are less suitable for analyses of deep phylogenies. Therefore only LSU sequences were applied in our phylogenetic studies. The LSU dataset was enriched with sequences published by Wang et al. (2004), Tomšovský et al. (2010), Tomšovský (2012), and Vlasák et al. (2012). Together, these sequences represent the main lineages of the *Polyporales*. Some sequences obtained from GenBank were also added to the respective LSU dataset. The sequence of *Thelephora palmata* (Scop.) Fr. was selected as an outgroup.

Sequences were aligned in Bioedit and adjusted by the Muscle web server (Anonymus on-line). Phylogenetic analyses were carried out in PHYML estimating maximum likelihood phylogenies and run at the server Phylogeny.fr (Dereeper et al. 2008) using the “A la Carte” mode. The alignment was treated with Gblock, eliminating poorly aligned positions and ambiguous regions, and the GTR substitution model was selected for the ITS dataset. Bootstrap branch support values (BP) were estimated in PHYML under the maximum likelihood criterion using the default number of 100 replicates.

## RESULTS

*Aurantiporus alborubescens* (Bourdot & Galzin) H. Jahn, Westfälische Pilzbriefe 9(6-7): 99, 1973. Figs. 1–4

On 25<sup>th</sup> June 2011, about 25 rather young basidiocarps of the species were discovered on both sides of an uprooted beech trunk in the western part of Holý kopec Nature Reserve. The trunk, measuring about 60 cm in diameter, was partly decorticated and moderately decayed [stage of decay 3(–4) according to the scale used by Heilmann-Clausen 2001] and located on a gentle NW slope approximately 70 m from the western edge of the reserve, at an altitude of 440 m. Several fruitbodies of *Fomes fomentarius* (L.) Fr. were observed on the same log (Fig. 1). The locality was subsequently visited several times during the season in order to obtain photographic documentation of the aging fruitbodies (Figs. 2–4). The last visit was carried out on 27<sup>th</sup> September, when large and well-developed as well as old drying basidiocarps were detected. The following year, five young fruitbodies were found on the same trunk at the end of July and, despite a long-lasting period of extremely dry weather, well-developed mature fruitbodies were observed at the end of September.

### Description

Fruitbodies annual (but rather well-preserved remnants of fruitbodies of the year 2011 observed in June 2012), pileate, sessile, unguulate or semicircular, often confluent with adjacent ones, up to 36 cm long, 12 cm wide and 14 cm thick. Upper surface irregularly tuberculate or dotted in places, usually roughly strigose with fasciculate fibrils, less hirsute to almost smooth when older, at first whitish, becoming pinkish cinnamon, old pink to flesh-coloured (similar to the colour of context of *Amanita rubescens* Pers. when cut). Tubes longest near the substratum, up to 20 mm long, incarnate beige, distinctly paler (whitish) near the pore surface. Pores round, small, white, producing numerous watery droplets when growing rapidly (Fig. 3). Context rather tough, but not very resilient, rather brittle,

juicy when young, concentrically zoned from the point of attachment and from the same place also radially fibrillose, dull pale pinkish to yellowish pinkish, contrasting with paler tubes. Taste acidulous, slightly unpleasant, rancid-soapy on the back of the tongue after a while. Smell very strong, penetrating, sweetish soapy, practically the same as in *Mycoacia nothofagi* (G. Cunn.) Nakasone and also similar to the smell of *Cortinarius traganus* (Fr.) Fr. An identical smell, sometimes even stronger, also emerges from the underlying rotting wood under the fruitbodies. Surface of pileus and pores as well as context slowly becoming dirty brownish orange with 30% KOH solution, after several hours developing into a flesh-pink or reddish pink spot with dirty greyish brownish (pinkish) margin. During drying the whole fruitbody darkens, shrinks considerably and becomes very hard and dense. Yellowish oily blotches remain on paper under drying fruitbodies.

Hyphal system monomitic. Contextual hyphae often agglutinated in bundles, cylindrical, thin- to very slightly thick-walled, fragile (often with refractive gelatinous prolapsing content when broken), with abundant clamps, smooth, 4–6  $\mu\text{m}$  wide. Hyphae in tubular trama thin-walled, cylindrical, clamped, 3–4  $\mu\text{m}$  wide. Basidia clavate, tetrasporic, thin-walled, 25–28  $\times$  7–8  $\mu\text{m}$ . Basidiospores smooth, ellipsoid, 5–6.5  $\times$  3.8–4.8  $\mu\text{m}$  (av. 5.8  $\times$  4.3  $\mu\text{m}$ ),  $Q = 1.2\text{--}1.5$  ( $Q_{\text{av}} = 1.35$ ), slightly thick-walled, inamyloid.

### DNA analysis

The sequencing of both ITS and LSU regions of rRNA gene were successful in *Aurantiporus alborubescens* and *A. croceus* (for details of *Aurantiporus* spp. sequences see Tab. 1). The obtained ITS sequence of *A. alborubescens* showed 98% identity to the sequence of *A. alborubescens* (AJ006683) published by Yao et al. (1999) using the BLAST algorithm (Altschul et al. 1990). The second most identical sequences were those of *Phlebia acanthocystis* (91%). In comparison, the ITS sequence of *Aurantiporus croceus* did not match any sequence from GenBank for more than 85%.

The aligned dataset of LSU sequences treated with Gblock was composed of 487 positions. Maximum likelihood analyses yielded trees with the following likelihood values and model parameters: ln = -3515.57540, invar: 0.556; f(A): 0.27223; f(C): 0.19124; f(G): 0.28771; f(T): 0.24882.

The results (Fig. 5) indicate that the two analysed species are not closely related to each other, neither to any of the taxa *Aurantiporus fissilis*, *Hapalopilus rutilans* (Pers.) P. Karst. or *Tyromyces chioneus* (Fr.) P. Karst.

### Specimens examined

Czech Republic. Moravia. Uherské Hradiště District, Chřiby hills, Buchlovice, Holý kopec Nature Reserve, western part, alt. 440 m, old beech forest, on fallen beech trunk, 27 June 2011, leg. J. Běťák, det. D. Dvořák (BRNU 627479); *ibid.*, 24 Sept. 2012, leg. et det. J. Běťák et D. Dvořák (BRNU 627480).





**Fig. 1.** Site of occurrence of *Aurantiporus alborubescens* in Holý kopec Nature Reserve on 25 June 2011. Note that several fruitbodies of the species in the middle of the left trunk are accompanied by perennial fruitbodies of *Fomes fomentarius* on the left. Photo J. Běťák.



**Fig. 2.** Group of fruitbodies of *Aurantiporus alborubescens*, Holý kopec Nature Reserve, 25 June 2011 (BRNU 627479). Photo J. Běťák.



**Fig. 3.** Group of several young fruitbodies of *Aurantiporus alborubescens*, Holý kopec Nature Reserve, 27 August 2011. Abundant ‘weeping’ indicates rapid development of the fruitbodies. Photo J. Běťák.



**Fig. 4.** Fruitbodies of *Aurantiporus alborubescens*, Holý kopec Nature Reserve, 27 August 2011. Note the roughly hirsute upper surface of the cap, which has already become rusty reddish. Photo J. Běťák.





## DISCUSSION

**Similar species and taxonomic position of *Aurantiporus alborubescens***

The discussed species was originally described as *Phaeolus albosordescens* var. *alborubescens* (Bourdot & Galzin 1925), and has been considered to be closely related to the similar and more widespread *Tyromyces fissilis* (Berk. & M.A. Curtis) Bondartsev & Singer by most recent authors. The morphological differences of both polypores are rather subtle – *T. fissilis* is usually less hirsute and more or less whitish and shows no significant reaction with KOH. However, older fruitbodies of *T. fissilis* may also become dirty pinkish. Moreover, the reaction with KOH in *Aurantiporus alborubescens* seems to vary; in our fruitbodies the context and surface becomes dirty reddish only slowly. In these cases, the main reliable distinguishing features of *A. alborubescens* are the strong sweet scent of fresh fruitbodies and considerably larger spores.

According to Núñez & Ryvarden (1999), also *Tyromyces transformatus* Núñez & Ryvarden, described from Japan and later combined into *Aurantiporus* (Zmitrovich et al. 2006), seems to be rather similar to *A. alborubescens*. It exhibits the same way of shrinking during drying and differs particularly in its small, thin-fleshed fruitbodies, very small pores, somewhat smaller basidia and also its occurrence on conifers. We did not have the opportunity to study material of this species.

Certain similarities of *A. croceus*, *Tyromyces fissilis* and *Phaeolus alborubescens* (Bourdot & Galzin) Bourdot & Galzin, such as the sappy context and difficult, lengthy drying accompanied by exsudate production, led Jahn (1973) to combine the two latter taxa into the genus *Aurantiporus* with *Polyporus pilotae* Schwein. [= *P. croceus* (Pers.) Fr.] as the type species. After detailed morphological comparison of *Hapalopilus nidulans* (Fr.) P. Karst (= *H. rutilans*) and *Aurantiporus croceus*, mainly on the basis of different anatomy and pigment solubility, the author questioned closer relations between these two species. Some other mycologists (Ryvarden 1991, Ryvarden & Gilbertson 1994, Bernicchia 2005) treated *Aurantiporus* as a taxonomic synonym of *Hapalopilus*, following the earlier opinion of Kotlaba & Pouzar (1966), while *A. fissilis* and *A. alborubescens* were placed in *Tyromyces*. However, in a DNA-based phylogenetic study of members of *Tyromyces* s. l. (Yao et al. 1999), it was demonstrated that *T. alborubescens* does not belong to *Tyromyces*.

Our results do not confirm close relations between *Aurantiporus alborubescens*, *A. croceus*, and *A. fissilis*. Moreover, none of these species seems to be congeneric with the type species of *Hapalopilus*, *Tyromyces*, *Climacocystis* or *Spongipellis*. Therefore the generic name *Aurantiporus* should only be applied to *A. croceus* (of the three *Aurantiporus* species mentioned by Jahn). The most closely related taxon (for which LSU sequences are available) of *A. alborubescens*



is *Ceriporiopsis pseudoplacenta* Vlasák & Ryvarden, recently described from western USA (Vlasák et al. 2012).

Our data partly disagree with the phylogeny of ribosomal SSU sequences performed by Ko et al. (2001). Their results revealed a considerable distance between the positions of *Aurantiporus alborubescens* and *A. croceus*, but *A. croceus* grouped with *Hapalopilus rutilans* (under the name of *H. nidulans*) in one clade. Nevertheless, the relationship between *Aurantiporus croceus* and *Hapalopilus rutilans* was not supported by a high bootstrap value. Moreover, the phlebioid clade species were insufficiently represented in the study.

Although evolutionary relations within the *Polyporales* have recently been studied (Binder et al. 2013, Ortiz-Santana et al. 2013), the phylogenetic data of some genera (including *Aurantiporus* and *Hapalopilus*) are incomplete. During the past few years the genera *Ceriporiopsis* and *Spongipellis* have been shown to be polyphyletic (Tomšovský et al. 2010, Tomšovský 2012) and in the above-mentioned more extensive phylogenies within the *Polyporales* neither some other traditional generic concepts can be maintained (Binder et al. 2013, Ortiz-Santana et al. 2013). In order to understand the taxonomy and phylogeny of *Aurantiporus* species, some other polypore species need to be sequenced and thoroughly studied. For example two boreal *Hapalopilus* species, *H. aurantiacus* (Rostk.) Bondartsev & Singer and *H. ochraceolateritius* (Bondartsev) Bondartsev & Singer, omitted or misunderstood by some authors (e.g. Ryvarden & Gilbertson 1993, Bernicchia 2005) but reintroduced and treated as a good species by Niemelä (2005), deserve a more detailed study. Also, the newly established species *Aurantiporus priscus* Niemelä, Miettinen & Manninen (Niemelä et al. 2012) is to be studied, since it shares many morphological similarities with *Ceriporiopsis pseudoplacenta*. Therefore we speculate that these two taxa may be closely related.

Until a sounder phylogeny of polyporoid members of the phlebioid clade is performed and necessary taxonomical changes are carried out, we prefer to keep the three studied species within the genus *Aurantiporus*.

**Note.** There are two orthographical variants of the genus name, *Aurantiporus* and *Aurantioporus*. The first one was used in the original genus description (Murrill 1905) and is therefore the name to be used (see McNeill et al. 2012, Art. 60 and 61) despite the fact that it is probably not linguistically correct. This conclusion is also in accordance with the MycoBank database ([www.mycobank.org](http://www.mycobank.org) – accessed 17 April 2014).

## Ecology and distribution

*Aurantiporus alborubescens* belongs to the strictest old-growth beech forest species in Europe. It is most probably a heart-rot species (Fritz et al. 2010), strongly preferring large standing or fallen trunks or snags of *Fagus sylvatica* as its substrate, but was allegedly also recorded on *Fraxinus excelsior* in England (Legon & Henrici on-line). However, we could not find a single record from

*Fraxinus* in the FRDBI database (Kirk & Cooper on-line). According to a personal communication by M. Ainsworth, the only collection from *Fraxinus* in fact originates from *Fagus sylvatica*, hence all European records with a known host come from *Fagus*. In Asia, besides occurrence on *Fagus* (Melik-Khachatryan & Martirosyan 1971), the species was reported to grow on *Populus tremula* and even on cultivated *Populus balsamifera* in SW Siberia (Arefyev 2004).

Although generally considered to be an excellent indicator species of natural old-growth beech forests on both national (Heilmann-Clausen & Christensen 2000, Ainsworth 2005, Fichtner & Lüderitz 2013) and European scale (Christensen et al. 2004), the Moravian find suggests that *Aurantiporus alborubescens* may also occur in forests with a relatively short history of spontaneous development. The Holý kopec Nature Reserve in the Chřiby hills was established in 1975 and has remained consistently unmanaged only since 1999. However, there is a large amount of decaying beech trunks in the reserve and also the diversity of lignicolous fungi is surprisingly high. For example, 15 out of 21 indicator species proposed by Christensen et al. (2004) to assess beech forest naturalness in Europe occur here, along with some other extremely rare species, such as *Ionomidotis irregularis* (Schwein.) E.J. Durand (Běťák et al. 2012).

Besides the type locality in central France in Fontainebleau forest (Bourdot & Galzin 1925), the species is known from several localities in England (Pegler & Legon 1993, Legon & Henrici on-line), Denmark (Koch 1975), N Germany (Amelang in litt., Lüderitz 2001) and Belgium (Walley on-line). In the past decade, new localities have been discovered in S Sweden (Fritz et al. 2010). The species apparently prefers lowland beech forests in (sub)oceanic areas – despite of the considerably large areas of old-growth beech forests with potentially favourable conditions for the species in Central and Eastern Europe, it had presumably not been known from the Carpathians nor from the Balkans (Adamčík et al. 2007). Occurrence in Žofínský prales Virgin Forest in Bohemia published by Christensen et al. (2004: 234) is undoubtedly an error.

Outside Europe, the species is reported from Armenia and Iran (Ghobad-Nejhad 2011, Melik-Khachatryan & Martirosyan 1971, Fallahyan 1973), Russian Caucasus (Ryvarden & Gilbertson 1994) and from two Russian provinces in SW Siberia (Kurganskaya Oblast and Tyumenskaya Oblast) (Bondartseva 1998, Arefyev 2008). The last mentioned localities are situated in a highly continental area far outside the main range of the species, while all other known occurrences in Europe and adjacent areas fall within the natural range of *Fagus sylvatica* L. (incl. *Fagus orientalis* Lipsky in the Caucasian region; Meusel & Jäger 1965). Since heart-rot fungi are thought to be highly selective in host tree species (Boddy & Heilmann-Clausen 2008) and the Siberian finds were from wood of *Populus*, we have some doubts about the identity of the Russian fungi. These collections may

in fact represent the similar *Aurantiporus fissilis* (which often occurs on *Populus* in Central Europe) or another species.

The occurrence of *Aurantiporus alborubescens* in SE Moravia indicates that the real range of climatic conditions allowing the existence of the species is broader than previously assumed (see e.g. Fritz et al. 2010 and Heilmann-Clausen & Walley 2007). In Europe, the Moravian locality is by far the most distant from the sea (compare with the distribution map in Fritz et al. 2010) and is characterised by a more continental climate and higher altitude in comparison to all other European sites. The new observation of the species in Central Europe may be considered an evidence of its recent spread in Europe caused by climate change (as also indicated by an increasing number of new localities in England and Sweden). However, we consider this unlikely, since most of the recent species expanding their distribution range in Central Europe spread from E Europe in more or less western direction – e.g. *Schizopora flavipora* (Berk. & M.A. Curtis ex Cooke) Ryvarden (Vampola 1993, Kotlaba 1994) or *Trichaptum bifforme* (Fr.) Ryvarden (Běťák, Dvořák pers. obs.). These observations suggests that the climate in Central Europe is becoming rather more continental. Another possible explanation is that the species may long have been overlooked, but this seems even less probable to us. The species has very conspicuous, rather long-lasting fruit-bodies which occur regularly over many years (Fritz et al. 2010) and has affinity to old-growth forest reserves, which are often relatively well surveyed. Thus, the observed recent changes in the known distribution of *Aurantiporus alborubescens* are possibly caused by more complex factors and may also be just accidental, stochastic in nature.

#### ACKNOWLEDGEMENTS

We are indebted to Martyn Ainsworth, Norbert Amelang, Masoomeh Ghobad-Nejhad and Jacob Heilmann-Clausen for valuable information and providing some papers. Numerous important comments of the reviewers are also highly acknowledged.

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