

# First records of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* (Boletales) in association with non-native *Fagaceae*, with taxonomic remarks

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**Abstract.** The paper reports and discusses some interesting findings of two southern boletes – *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* – in association with the non-native for Europe *Quercus rubra* (Northern Red Oak). Descriptions and illustrations of the studied collections are provided, along with molecular phylogenetic analysis of the ITS sequences, confirming their affiliation. These are the first records of *Exsudoporus* and *Pulchroboletus* species with this host-tree. An overview of earlier reports of boletes with tubulate hymenophore with *Q. rubra* in Europe is provided and compared to data from North America. The authors also report another character for setting apart *E. permagnificus* from the members of the genus *Butyriboletus*, an earlier suggested affinity. Some findings related to the distinction of the European members of *Pulchroboletus* and *Alessioporus* are also brought forward.

**Key words:** *Boletus*, invasive trees, non-native mycorrhizal hosts, *Quercus borealis*, *Xerocomus*

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## Introduction.

*Exsudoporus permagnificus* (Pöder) Vizzini, Simonini & Gelardi and *Pulchroboletus roseoalbidus* (Alessio & Littini) Gelardi, Vizzini & Simonini are uncommon bolete species, with a range known to span out mostly across the Mediterranean area of Europe (Assyov 2005; Muñoz 2005; Assyov & Denchev 2009; Gelardi & al. 2014; Sarkina 2016). They are not common in Bulgaria and were recorded earlier from a few localities along the Black Sea Coast (Assyov 2005; Assyov & Denchev 2009). In 2013, the second author received from a correspondent some specimens of both species found in a locality in Southeast Bulgaria. They were reported by the collector to have been found under *Quercus rubra* L. (Northern Red Oak). This is a North American oak

species, introduced in Europe late in the 17<sup>th</sup> century (Nyssen & al. 2016) and now known as possessing invasive properties (Woziwoda & al. 2014). Parasitic and saprotrophic (especially wood-destroying) fungi on *Q. rubra* were subject to interest in Europe, including in Bulgaria (Domański 1982; Kehr 1992; Marçais & Delatour 1996; Sameva & al. 2009; Rossnev & al. 2010; Stoykov 2012, 2017). Ectomycorrhizal fungi related to the Northern Red Oak in Europe are still less known, but studies focused on its stands have been published so far from a few countries (Sammler 2004; Gebhardt & al. 2007; Malanyuk 2014). Thus, the findings of *E. permagnificus* and *P. roseoalbidus* in relation to this tree are very interesting and further well-documented collections of both boletes were obtained in 2014, after a thorough survey of the same locality, conducted by

one of the authors. Those findings are described and discussed herein.

## Material and methods

Materials used in this study were collected in 2013 and 2014 and preserved in air-dried state in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF). Methods of morphological study were described in detail in Assyov (2012). Microscopic observations were held with AmScope T360B light microscope equipped with AmScope MU900 digital camera. All measurements were made on digital images with the aid of Piximetre v. 5.9. Descriptions provided in the text are solely based on specimens, associated with *Q. rubra*. The colour notations adhere as far as possible to the *Flora of British Fungi Colour Identification Chart* (Anonymous 1969).

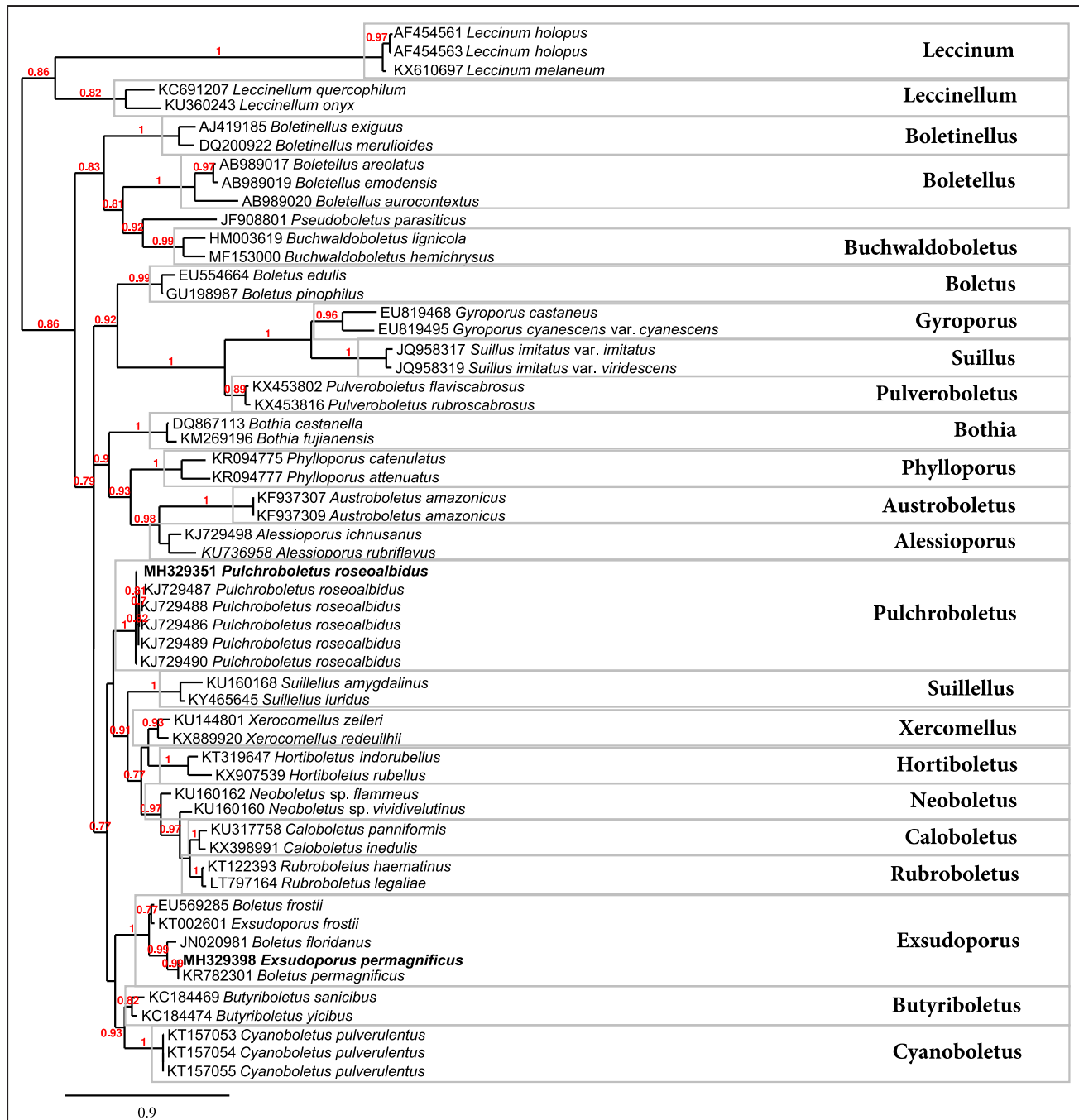
DNA was also isolated from dried samples, following the procedures of Eurx Genematrix Plant & Fungi DNA Purification Kit with small modifications, namely increasing the concentration (100 mg mL<sup>-1</sup> and 10 mg mL<sup>-1</sup>) and the volume (10 µL) of RNase A and proteinase K, respectively (Bozok 2016). Primers ITS1F-ITS4 were used for amplification of the ITS rDNA region in PCR (White & al. 1990). PCR conditions were as follows: 94°C for 5 min, followed by 30 cycles for 30 s at 94°C, 45 s at 53°C, 60 s at 72°C, and a final extension of 10 min at 72°C. PCR amplifications were verified by electrophoresis on a 1.5% agarose gel and then DNA sequence analyses of successful amplifications were made by BigDye Terminator v3.1 Sequencing Kit, using again the ITS1F-ITS4 primers. ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA, US) was used for running the sequencing reactions. Raw sequence chromatograms were edited and aligned using Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA). A phylogenetic tree was obtained by using Maximum Likelihood Analysis in PhyML 3.1/3.0 aLRT (Guindon & al. 2008) with GTR+I model of evolution. Furthermore, SH-aLRT test in PhyML was used to calculate the branch support (Anisimova & Gascuel 2006). The phylogenetic tree was drawn by using TreeDyn 198.3(doc) (Chevenet & al. 2006). The sequences obtained from this study were deposited in GenBank as accessions MH329398 for *E. permagnificus* and MH329351 for *P. roseoalbidus*.

The systematics of the European species of *Boletaceae* discussed in the paper follows closely the recent phylogenetically derived development, an excellent summary of which could be seen in Moreau & al. (2015a). For the phylogenetic placement of the extra-European taxa mentioned in the text, the reader is referred to Drehmel & al. (2008), Dentinger & al. (2010), Moreau & al. (2013), Nuhn & al. (2013), Arora & Frank (2014), Wu & al. (2014, 2016a, b) and Zhao & al. (2015).

## Results

### Molecular phylogenetic analysis

In the presented study, occurrence of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* (*Boletaceae*, *Boletales*) in association with non-native *Fagaceae* Dumort. was documented for the first time. For this reason, molecular studies have been performed to prove the accuracy of the species identification, as achieved by means of morphological analysis. Phylogenetic assessment of ITS rDNA sequences was preferred considering the availability of reference sequences in public databases of the concerned taxa. Phylogenetic position of *E. permagnificus* and *P. roseoalbidus* was verified by comparing the ITS rDNA sequences obtained from this study with the sequences selected from GenBank (Fig. 1, Table 1). The sequences KJ729486, KJ729487, KJ729488, KJ729489, and KJ729490 submitted to the GenBank by Gelardi & al. (2014) were used for comparison with our *P. roseoalbidus*. The one from our specimen nests in a clade, with the above-mentioned five, including KJ729486 from the epitype, and the identity of our collection was thus indisputably verified. For the comparison of our *E. permagnificus* collection, we were able to assess a single unpublished sequence (KR782301) submitted to GenBank by Bellanger, Moreau and Richard as contributions to a molecular database of the Mediterranean fungi. The sequences of the other species presented in Table 1 and used in Fig. 1 have been added, so that the phylogenetic location of the discussed species could be established more precisely. In the ITS phylogeny *Pulchroboletus* Gelardi, Vizzini & Simonini appears as a well-supported clade, related to *Suillellus* Murrill, *Xerocomellus* Šutara, *Hortiboletus* Simonini, Vizzini & Gelardi, *Neoboletus* Gelardi, Simonini & Vizzini, *Caloboletus* Vizzini, and



**Fig. 1.** Phylogenetic tree of the ITS sequences of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* obtained by Maximum Likelihood Analysis in PhyML (Substitution model: GTR + I, Gamma shape parameter: 1.333, Number of categories: 4, Proportion of invariant: 0.125). Maximum Likelihood Bayesian Inference posterior probabilities > 0.70 are shown. Accessions generated in this study are shown in bold.

*Rubroboletus* Kuan Zhao & Zhu L. Yang, and distant from the other member of the Hypoboletus group (Nuhn & al. 2013), where both genera are known to belong (Gelardi & al. 2014). The three members so far assigned to *Exsudoporus* Vizzini, Simonini & Gelardi also form a clade with a good support, related to those containing members of *Butyriboletus* D. Arora & J.L.

Frank and *Cyanoboletus* Gelardi, Vizzini & Simonini. The collections of both species reported here are the first from Southeast Europe, which identity is confirmed by means of molecular methods. Our phylogenetic analysis also demonstrates that Balkan sequences show no significant differences from the so far available reference sequences from other parts of Europe.

Table 1. Sequences from GenBank, included in the phylogenetic analyses.

Taxon	Origin	Length (bp)	GenBank Code	Reference
<i>Alessioporus ichnusanus</i>	France	768	KJ729498	Gelardi & al. (2014)
<i>Alessioporus rubriflavus</i>	USA	746	KU736958	Frank & al. (2017)
<i>Austroboletus amazonicus</i>	Colombia	660	KF937307	da Marcela & al. (2014)
<i>Austroboletus amazonicus</i>	Colombia	660	KF937309	da Marcela & al. (2014)
<i>Boletellus areolatus</i>	Japan	1057	AB989017	Sato & Hattori (2015)
<i>Boletellus emodensis</i>	Japan	1044	AB989019	Sato & Hattori (2015)
<i>Boletellus aurocontextus</i>	Japan	1078	AB989020	Sato & Hattori (2015)
<i>Boletinellus exiguus</i>	Brazil	657	AJ419185	Martin & Raidl (2002)
<i>Boletinellus merulioides</i>	unavailable	649	DQ200922	Matheny & al. (2007)
<i>Boletus edulis</i>	Spain	745	EU554664	Agueda & al. (2008)
<i>Boletus floridanus</i>	USA	691	JN020981	Dentinger & al. (2011)
<i>Boletus frostii</i>	USA	664	EU569285	Morris & al. (2008)
<i>Boletus permagnificus</i>	unavailable	759	KR782301	unpublished
<i>Boletus pinophilus</i>	Finland	722	GU198987	Korhonen & al. (2009)
<i>Bothia castanella</i>	USA	712	DQ867113	Halling & al. (2007)
<i>Bothia fujianensis</i>	China	697	KM269196	Zeng & al. (2015)
<i>Buchwaldoboletus lignicola</i>	Italy	687	HM003619	unpublished
<i>Buchwaldoboletus hemichyrsus</i>	USA	641	MF153000	unpublished
<i>Butyriboletus sanicibus</i>	China	787	KC184469	Arora & Frank (2014)
<i>Butyriboletus yicibus</i>	China	808	KC184474	Arora & Frank (2014)
<i>Caloboletus panniformis</i>	China	762	KU317758	unpublished
<i>Caloboletus inedulis</i>	USA	717	KX398991	unpublished
<i>Cyanoboletus pulverulentus</i>	Italy	697	KT157053	Gelardi & al. (2015)
<i>Cyanoboletus pulverulentus</i>	Portugal	689	KT157054	Gelardi & al. (2015)
<i>Cyanoboletus pulverulentus</i>	Italy	691	KT157055	Gelardi & al. (2015)
<i>Exsudoporus frostii</i>	USA	544	KT002601	unpublished
<i>Gyroporus castaneus</i>	USA	666	EU819468	Palmer & al. (2008)
<i>Gyroporus cyanescens</i> var. <i>cyanescens</i>	USA	640	EU819495	Palmer & al. (2008)
<i>Hortiboletus indorubellus</i>	India	718	KT319647	Chakraborty & al. (2016)
<i>Hortiboletus rubellus</i>	Pakistan	779	KX907539	Sarwar & al. (2016)
<i>Leccinellum quercophilum</i>	USA	830	KC691207	Kuo & al. (2013)
<i>Leccinellum onyx</i>	China	1080	KU360243	Li & al. (2016)
<i>Leccinum holopus</i>	Netherlands	1434	AF454561	Den Bakker & al. (2004)
<i>Leccinum holopus</i>	Sweden	1482	AF454563	Den Bakker & al. (2004)
<i>Leccinum melaneum</i>	Poland	927	KX610697	Kalucka & al. (2016)
<i>Neoboletus</i> sp. <i>flammeus</i>	USA	644	KU160162	unpublished
<i>Neoboletus</i> sp. <i>vividivelutinus</i>	USA	628	KU160160	unpublished
<i>Phylloporus catenulatus</i>	Bangladesh	691	KR094775	Hosen & Li (2017)
<i>Phylloporus attenuatus</i>	Bangladesh	798	KR094777	Hosen & Li (2017)
<i>Pseudoboletus parasiticus</i>	Italy	652	JF908801	Osmundson & al. (2013)
<i>Pulchroboletus roseoalbidus</i>	Italy	785	KJ729487	Gelardi & al. (2014)
<i>Pulchroboletus roseoalbidus</i>	Italy	771	KJ729488	Gelardi & al. (2014)
<i>Pulchroboletus roseoalbidus</i>	Italy	771	KJ729486	Gelardi & al. (2014)
<i>Pulchroboletus roseoalbidus</i>	Italy	773	KJ729489	Gelardi & al. (2014)
<i>Pulchroboletus roseoalbidus</i>	Italy	778	KJ729490	Gelardi & al. (2014)
<i>Pulveroboletus flaviscabrosus</i>	China	611	KX453802	Zeng & al. (2017)
<i>Pulveroboletus rubroscabrosus</i>	China	620	KX453816	Zeng & al. (2017)
<i>Rubroboletus haematinus</i>	USA	756	KT122393	unpublished
<i>Rubroboletus legaliae</i>	Czech Republic	738	LT797164	Janda & al. (2017)
<i>Suillellus amygdalinus</i>	USA	682	KU160168	unpublished
<i>Suillellus luridus</i>	unavailable	649	KY465645	unpublished

Table 1. Continuation.

Taxon	Origin	Length (bp)	GenBank Code	Reference
<i>Suillus imitatus</i> var. <i>imitatus</i>	USA	703	JQ958317	Nguyen & al. (2012)
<i>Suillus imitatus</i> var. <i>viridescens</i>	USA	703	JQ958319	Nguyen & al. (2012)
<i>Xerocomellus zelleri</i>	USA	684	KU144801	unpublished
<i>Xerocomellus redeuilhii</i>	Italy	654	KX889920	Simonini & al. (2016)

The names presented in the first column are the original names under which the accessions were deposited in the GenBank.

### Description of the collections

*Exsudoporus permagnificus* (Pöder) Vizzini, Simonini & Gelardi, Index Fungorum 183: 1, 2014 (Fig. 2a, b)

Basidiomata single or often clustered in groups of 3–8. Pileus up to 10 cm in diameter, convex to flat-convex, subsequently often flat or depressed, slightly felty or somewhat fibrillose, mat or smooth, often shiny, scarlet, red to blood-red, often paler towards the margin, tending to orange or luteous in places, in old basidiomata often fading partly or completely to olivaceous, blueing strongly when bruised; pileal margin straight or irregularly undulate, often upturned. Stipe up to 7 × 3 cm, cylindrical, usually tapering or swollen at the base, to narrowly clavate, straight or curved, usually ± rooting, light lemon-yellow, lemon-chrome or straw, downwards somewhat pale-coral, blood-red or purple-red at the same base,

with well developed red reticulum; stipe surface quickly and strongly blueing at the slightest touch. Context yellowish to off-white in the pileus, lemon-yellow in the stipe, blueing strongly when exposed to air, then fading to dingy or dirty-reddish orange. Tubes up to 1.5 cm long, adnate or subdecurrent, yellow, blueing when injured. Pores in young basidiomata red, subsequently orange-red or orange-yellow, usually paler at the cap margin, blueing when bruised, exuding yellow drops of liquid when young. Odour not distinctive. Taste somewhat acid. Basidiospores (12.8–) 14.5±0.7 (–16.3) × (5.9–) 6.6±0.4 (–7.8) μm (n=50), ratio (2.0–) 2.2±0.1 (–2.4), with 1–3 large guttules. Basidia mostly 4-spored, but occasionally 3- and 2-spored basidia occur, 34.2–43.9 × 9.8–11.8 μm, clavate. Cystidia scarce, 52–77 × 7.5–12 μm. Pileipellis a trichodermium of interwoven branched septate hyphae of loosely connected elements. Microchemical



Fig. 2. Collections of *Exsudoporus permagnificus* and *Pulchrobolus roseoalbidus*: **a** – *E. permagnificus* – basidiomata in situ; **b** – *E. permagnificus* – amyloid reaction of hyphae of stipe base context with Melzer's reagent; **c** – *P. roseoalbidus* – basidiomata in situ; **d** – *P. roseoalbidus* – fleeting amyloid reaction in hymenium at different magnification; arrows point to hymenophoral trama, where the reaction is easily discernible. Figures not to scale.

reactions: Melzer's solution with the hyphae of the pileus and the pileipellis reddish-brown, with stipe tissues distinct, slowly fading amyloid reaction observed (Fig. 2b).

Specimens examined: Thracian Lowland, Haskovo town, Kenana Park, 41°56'54.2"N, 25°32'23.3"E, 10.07.2013, E. Nankova (SOMF 29919, GenBank MH329398); idem, 14.07.2013, N. Apostolov (SOMF 29975); idem, 23.07.2014, B. Assyov (SOMF 29976).

*Pulchroboletus roseoalbidus* (Alessio & Littini) Gelardi, Vizzini & Simonini, in Gelardi, Simonini, Ercole & Vizzini, *Mycologia* 106(6): 1176, 2014 (Fig. 2c, d)

Basidiomata single or often clustered in groups of 3–9. Pileus up to 10 cm in diameter, initially hemispherical, then convex, finally flat-convex, flat or slightly depressed, often irregular, finely tomentose, then glabrous or somewhat fibrillose, occasionally finely cracked, at first often whitish, usually with a pale-pinkish tint or pinkish or reddish spots, subsequently tending towards pale-dull-rose all over, occasionally nearly entirely red, often developing pale-buff or buff tints, occasionally spotted with brick colour; surface unchanging, when bruised; margin flat to distinctly undulate and sometimes slightly appendiculate. Stipe up to 8 × 2 cm, tapering or spindle-shaped, straight or curved, somewhat radicating, seldom almost cylindrical or narrowly clavate; background yellowish-white, straw or pale-yellow, usually discolouring with age towards cream or off-white, with fine reddish rose, coral or scarlet granules, discolouring brownish or similar to the stipe background colours; stipe surface not blueing or blueing after rough handling. Reticulum absent, but a ring-like pattern of coarse granules is often present on the stipe surface. Context lemon-yellow or straw-yellow in the stipe, with age becoming off-white, paler in the cap and at least above the tubes and below the pileipellis pinkish or livid vinaceous, but often pinkish overall in the pileus, occasionally pinkish tints develop in the lower stipe context, blueing when exposed to the air; the autoxidation reaction is usually more uniform and paler in the pileus and deeper in the stipe, but sometimes it could be patchy or not develop at all. Tubes up to 1.5 cm long, adnate or subdecurrent, lemon-yellow when young, at maturity approaching lemon-chrome and subsequently with a slight olivaceous tint, blueing when injured. Pores angular, concolorous, blueing when bruised. Odour

not distinctive. Taste mild. Basidiospores broadly ellipsoid, (12.9–) 15.3±1.1 (–17.8) × (6.4–) 7.2±0.4 (–7.8) μm ( $n=50$ ), ratio (1.8–) 2.1±0.2 (–2.5), with 1–3 large guttules. Basidia clavate, generally 4-spored (2- and 3-spored basidia also occur), 27–40.5 × 10–15 μm. Cystidia 37–52.5 × 10–12 μm. Pileipellis a trichodermium of interwoven branched septate hyphae. Macrochemical and microchemical reactions: weak “fleeting-amyloid” reaction observed in the hymenophore (Fig. 2d), no other macro- or microchemical reaction has been noted.

Specimens examined: Thracian Lowland, Haskovo town, Kenana Park, 41°56'54.2"N, 25°32'23.3"E, 10.07.2013, E. Nankova (SOMF 29920, GenBank MH329351); idem, 23.07.2014, B. Assyov (SOMF 29977).

### Survey of the *Quercus rubra* stand

The stand, where the basidiomata of *E. permagnificus* and *P. roseoalbidus* were collected (Fig. 3), is situated in the urban Kenana Park in Haskovo town, South Bulgaria, on the average at 235 m a.s.l. The artificial afforestation with *Quercus rubra* covers an area of approximately 1 ha, developed on calcareous vertisols on an east-facing slope, with inclination less than 5%. The stand is isolated from all sides by broad and solid tarmac alleys. The stand itself is a pure plantation of *Q. rubra*, 40 years of age, according to the Park's management plan. Numerous seedlings of this species of different age are also present, resulting from natural reproduction by seeds *in situ*, with at least some individuals overpassing age of 10 years. Sparse young individuals of *Fraxinus ornus* L. are also present in the area, along with solitary young shrubs of *Crataegus monogyna* Jacq., *Rosa* sp. and *Rubus* sp. Herbaceous vegetation is sparse and includes mostly ruderal species, e.g. *Alliaria petiolata* (M. Bieb.) Cavara & Grande and *Geum urbanum* L. No native oak species, either mature or seedlings, have been found on site, but *Quercus cerris* L. and *Q. virgilliana* (Ten.) Ten. are among the dominant species in the nearby areas. The closest individuals of native oaks are situated at least 20 m away from the spots, where bolete basidiomata were found, but they seem well isolated, separated by the solid tarmac alleys. Moreover, several spots of boletes have been located up to 50 m from the potential native host trees. Mycorrhizal relationship with *Q. rubra* is thus the only feasible explanation.



**Fig. 3.** Studied site of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* in the stand of *Quercus rubra* at Kenana Park (Haskovo town, Bulgaria).

## Discussion

Identity of the above-described collections was initially established by morphological approach. The studied specimens of *E. permagnificus* from stands of *Q. rubra* matched perfectly the original description (Pöder 1981), as well as those presented in Icard & Hurtado (1997), Galli (1998), Lannoy & Estadès (2001), Lunghini & Perrone (2002), Estadès & Lannoy (2004), Muñoz (2005) and Šutara & al. (2009). They also agreed well with the earlier studied specimens from Bulgaria (Assyov 2005, 2017), albeit some differences in the spore parameters. Nevertheless, these were more or less within the range of variability known from the other parts of the geographic range. The specimens of *P. roseoalbidus* were congruent with the original description (Alessio 1987, but see comments in Assyov & Denchev 2009), as well as the ones presented in later sources on the species (Galli 1998; Lannoy & Estadès 2001; Ladurner & Simonini 2003; Estadès & Lannoy 2004; Šutara & al. 2009; Gelardi & al. 2014). Analysis of the ITS sequences of selected specimens confirmed the identity of both species as resolved by morphological approach. Furthermore, absence of significant differences suggested a lack of geographic divergence within the two species in question, although the geographic coverage is so far

scarce and involving more sequences in the future from both the East and the West Mediterranean is certainly interesting.

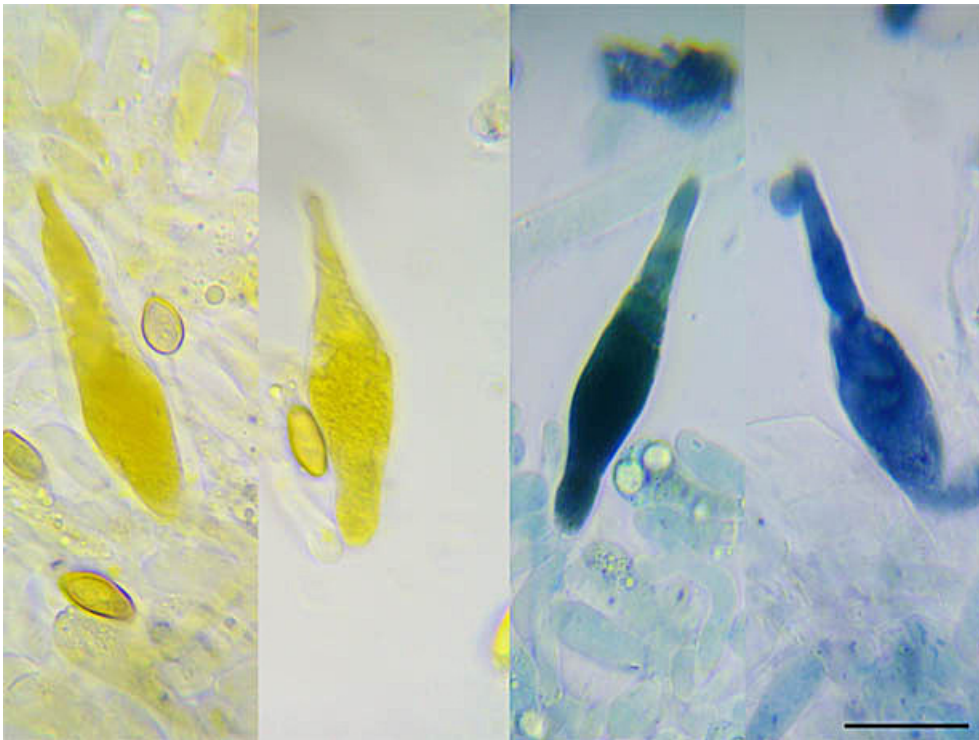
While the generic status of *Exsudoporus* was disputed by Wu & al. (2016b) and a possible synonymy with *Butyriboletus* was discussed, given the sharply distinct morphological characters, shared by all three currently known members of the *Exsudoporus* clade, the authors preferred rather to follow Vizzini and co-authors (in Vizzini 2014), at least for the time being. Furthermore, as seen from the description above, the authors have observed conspicuous violet, slowly fading amyloid reaction in the stipe tissues of *E. permagnificus*, of intensity similar to the one in *Caloboletus calopus* (Pers. : Fr.) Vizzini (Fig. 2b). We tend to interpret it as true positive amyloid reaction, rather than fleeting. This reaction is controversially reported in the literature on this species (Pöder 1981; Lannoy & Estadès 2001; Assyov 2005; Muñoz 2005). However, repeated testing of the sequenced and non-sequenced specimens at our disposal invariably yielded positive reaction. Positive amyloidity is generally not common in boletes and has thus been long considered as being of proven taxonomic value (Watling 1971). It is a well-known feature of the genus *Suillellus* and presents undoubted interest also in *Caloboletus*. To our knowledge, it has not been seen so far in any member of *Butyriboletus* (Smith & Thiers

1971; Muñoz 2005; Watling & Hills 2005; Assyov 2012; Arora & Frank 2014; Zhao & al. 2015; Bessette & al. 2016; Wu & al. 2016b), but here this calls for caution, as some bolete genera are known to encompass both members with positive and negative amyloidity, notably the above-mentioned *Caloboletus*. Although not uniformly distributed among the mentioned genera and in spite of the fact that no information could be found in literature on this reaction in *E. floridanus* (Singer) Vizzini, Simonini & Gelardi (Singer 1947, 1977; Bessette & al. 2016; Wu & al. 2016b), fleeting-amyloid reaction was reported earlier in *E. frostii* (J.L. Russell) Vizzini, Simonini & Gelardi (Smith & Thiers 1971). Therefore, the authors maintain that the presence of amyloidity in *E. permagnificus* merits attention as contributing to the reappraisal of this genus, whose three members cluster in a sister, but distinct clade to *Butyriboletus* s. str. in ITS-phylogeny (Fig. 1). While ITS may not necessarily reflect the infraspecific relationships, the clustering of the three species of *Exsudoporus* certainly merits attention. Although a definite answer could be given only after a multigene phylogenetic analysis including all currently assigned members of *Exsudoporus*, the authors urge those in possession of representative specimens of *E. floridanus* and *E. frostii* to reevaluate the reaction with Melzer's reagent and report it in a suitable manner, so that it could help the future judgement of the generic status of *Exsudoporus* and the morphological circumscription of the related entities. Along these lines, the presence of stuffed pores should be also traced out further, for such pores were not seen by us in our specimens of *E. permagnificus*, nor were they described in the earlier studies, mentioned above. This peculiarity was already found to occur in four clades of *Boletaceae*, with the warning that it should be interpreted in combination with other characteristics (Wu & al. 2014), which is also true for the amyloid reaction when tackling taxa of generic level.

The morphological features of *P. roseoalbidus* are thoroughly documented in the European mycological literature (Alessio 1987; Arenal & al. 1995; Galli 1998; Lannoy & Estadès 2001; Ladurner & Simonini 2003; Estadès & Lannoy 2004; Assyov & Denchev 2009; Šutara & al. 2009; Gelardi & al. 2014). The species is well known for its secondary angiocarpic development, a rare feature, in the European boletes solely known in the related *Alessioporus ichnusanus* (Alessio, Galli & Littini) Gelardi, Vizzini & Simonini from the suprageneric clade of *Hypoboletus*. Initially

proposed as monotypic, the genus *Pulchroboletus* was recently shown to contain at least one more species, *P. rubricitrinus* (Murrill) Farid & A.R. Franck, spread in the eastern United States (Farid & al. 2017). While the North American *Pulchroboletus* also shows prominent bright colours, no secondary angiocarpy has been recorded so far in this species. Furthermore, mention deserves the discovery of a second *Alessioporus* Gelardi, Vizzini & Simonini, *A. rubriflavus* J.L. Frank, A.R. Bessette & A.E. Bessette, again in the eastern North America (Frank & al. 2017). This notable species is also characterized by vivid colours and does not show secondary angiocarpic development, thus rendering the initially very obvious morphological difference of the two sister genera somewhat obsolete, although the secondary angiocarpy is apparently a homoplastic feature. However, both species of *Alessioporus* possess reticulate or longitudinally striate stipe surface, normally not present in the two members of *Pulchroboletus*. While the generic status of *Alessioporus* and *Pulchroboletus* seems presently undisputed, their morphological distinction still may benefit from additional separating characters. Pseudocystidia as cheilocystidia, turning golden-yellow in KOH have been seen in some collections of *A. ichnusanus* (Assyov & Stoykov 2011), but not in others (Gelardi & al. 2014). They are scarce, but nevertheless very striking (Fig. 4) and have been observed repeatedly by the second author of this paper. No cheilocystidia seem to have been found in *A. rubriflavus* by Frank & al. (2017) and the pleurocystidia of the species have been described as hyaline in KOH. Pseudocystidia have never been observed in *P. roseoalbidus* (Ladurner & Simonini 2003; Assyov & Denchev 2009; Gelardi & al. 2014). Farid & al. (2017) reported "light-brownish to hyaline in KOH" cheilocystidia in the holotype of *P. rubricitrinus*. Cystidia with prominent yellow pigments are known in several members of the phylogenetically related genus *Aureoboletus* Pouzar (Wu & al. 2016b). However, the yellow pigments there seem of apparently different nature, as they tend to dissolve in KOH. As far as pseudocystidia are a rather rare feature at least in the European boletes, we feel that this character merits further observation in all known members of *Alessioporus* and *Pulchroboletus*. Wu & al. (2014) found a peculiar, particularly fine ornamentation pattern, termed "pinpricks", in the basidiospores of some members of the *Hemileccinum/Corneroboletus* lineage (the latter genus recently





**Fig. 4.** Pseudocystidia in *Alessioporos ichnusanus* in squash mounts: on the left – two cystidia in KOH demonstrating bright- yellow content; on the right – two cystidia treated in KOH, followed by addition of aqueous Cotton blue (selective absorbance of the dye by the yellow-pigmented cystidial content is easily seen). Illustration based upon specimen SOMF 29981 (Bulgaria, Emona village, 08.09.2014). Scale bar = 20  $\mu$ m.

merged into *Hemileccinum* Šutara by Wu & al. 2016b), together with *Aureoboletus* shown to be related to *Alessioporos* and *Pulchroboletus* (Gelardi & al. 2014; Wu & al. 2016b). The two European members of the latter genera have been studied by means of SEM, but no structures similar to pinpricks have been noted or could be seen in the published SEM photographs (Oolbekkink 1991; Arenal & al. 1995; Assyov & Stoykov 2011); pinpricks were said to be discernible at magnifications exceeding 20000 $\times$  (Wu & al. 2014). The attempts of one of the authors to detect such type of ornamentation in *A. ichnusanus* or *P. roseoalbidus* did not yield any satisfactory results to this very moment due to technical reasons. Dedicated observations are still necessary in *Alessioporos* and *Pulchroboletus* so as to prove that this peculiar ornamentation is indeed absent, for this would further delineate them from other related genera of *Xerocomoideae* Singer. Apart from the so far discussed features, Farid & al. (2017) have identified three sequences from earlier environmental samplings as belonging to the *Pulchroboletus* clade, thus suggesting the existence of yet unidentified species in this genus. One of those sequences comes from a bright-orange sclerotium (GenBank FJ480444). It was generated by study of Smith & Pfister (2009) on tuberculate ectomycorrhizae in *Boletus rubropunctus* Peck, sclerotia-forming species of unresolved

placement, but appearing in ITS phylogeny in Smith & Pfister (2009) as a sister clade to *Aureoboletus*, a genus residing in the *Hypoboletus* group in Nuhn & al. (2013). Sclerotia in *Boletales* are notably present in the more distant lineages, such as *Hygrophoropsidaceae* Kühner, *Paxillaceae* Lotsy and *Sclerodermataceae* Corda (Agerer 1999, Smith & al. 2015). Indeed, such structures have not been described yet in the species so far assigned to *Alessioporos* and *Pulchroboletus*, and targeted observations of the below-ground features in those genera would be undoubtedly of value.

The observed locality of *E. permagnificus* and *P. roseoalbidus* is located in a large parkland with urban backdrop. The fungi were found in a relatively isolated area planted with *Q. rubra*. The distance of basidiomata of *E. permagnificus* and *P. roseoalbidus* in the *Q. rubra* stand from areas with native oak species ranged from 20 m to 50 m. To our knowledge, no studies of the extent of genets in those two genera exist at present, but the mentioned distances considerably exceed the genet sizes reported in other boletes by Dahlberg & Stenlid (1994), Selse (2003), Burchhardt & al. (2011) and Lee & Koo (2016). Observations have shown that no other trees, except seedlings of *F. ornus*, are present in the stand, thus unequivocally pointing out at the Red Oak as the only available host. The former species was ruled out as a possible host as no ectomycorrhizae have

been identified with this tree (De Roman & al. 2005), but only arbuscular mycorrhizae instead, similarly to most other so far studied *Oleaceae* Hoffmans. & Link (Maremmani & al. 2003, Anonymous 2011). *Quercus rubra* is an oak species originating from North America and used in afforestation and landscape gardening in Bulgaria. It is known to propagate well under local climate by seeds and in the locality of the Kenana Park self-seeding was also observed. The exact origin of the mycorrhiza is unknown, but presumably inoculation of seedlings with *E. permagnificus* and *P. roseoalbidus* might have occurred spontaneously in nurseries, where the Red Oak material was produced or, even more likely, it could have evolved on the place after sowing of acorns *in situ*. This second possibility is especially feasible as this is the most common way of larger-scale oak reproduction employed in Bulgaria, and both boletes are present in adjacent areas, dominated by native oak species. However, despite our attempts, we were unable to trace out the origin of plants in the stand.

The finding of *E. permagnificus* and *P. roseoalbidus* is an interesting fact, as the artificial stands of *Q. rubra*, which are widespread in the country, do not show high diversity of mycorrhizal basidiomycetes and boletes in particular (Assyov, unpubl. obs.), although the studies in other European countries have shown a number of ectomycorrhizal fungi related to this host-tree. Few earlier observed boletes in such plantations in Europe are *Boletus edulis* Bull. : Fr., *Boletus reticulatus* Schaeff., *Butyriboletus appendiculatus* (Schaeff.) D. Arora & J.L. Frank, *Chalciporus piperatus* (Bull. : Fr.) Bataille, *Hortiboletus rubellus* (Krombh.) Simonini, Vizzini & Gelardi, *Imleria badia* (Fr. : Fr.) Vizzini, *Suillellus luridus* (Schaeff. : Fr.) Murrill, *Tylopilus felleus* (Bull. : Fr.) P. Karst., and *Xerocomellus chrysenteron* s. l. (Tab. 2). In Bulgaria, only the second was found once in Red Oak plantations (Assyov 2004). The reasons behind this are so far unclear and there have been no above- or below-ground studies in the country on this topic. One possible explanation, however, could be the fact that *Q. rubra*, apart from ectomycorrhiza, is known to develop vesicular-arbuscular mycorrhiza, similarly to other oaks of section *Lobatae* Loudon (Dickie & al. 2001). A few other bolete species have been recorded in Europe in the presence of the Red Oak, but in mixed stands where the host-tree could not be identified with certainty – *Boletus aereus* Bull. : Fr., *Leccinum aurantiacum* (Bull.) Gray, *Phylloporus pelletieri* (Lév.) Quél., *Rheubarbariboletus armeniacus* (Quél.) Vizzini, Simonini & Gelardi,

*Xerocomellus ripariellus* (Redeuilh) Šutara, and *Xerocomus subtomentosus* (L. : Fr.) Quél. (Volders 1996, Wilga 2000, Cavet & Martin 2008, Fernández-Vicente & al. 2008, Muñoz & al. 2008, Tsylyryk & al. 2010, Malanyuk 2014; records of species, already present in Table 2 are excluded from the above list).

The presumed alteration of hosts is also notable, for it involves members of different oak clades (Manos & al. 1999, Simeone & al. 2016) – section *Lobatae* (*Q. rubra*) and section *Virentes* Loudon and the *Cerris* group (native species with which the two fungal species are found in the area). Such host-switch is feasible, as the *Lobatae* group was shown to be phylogenetically closer related to the *Quercus* group (including *Q. virgilliana*) than to the groups including *Q. cerris* and the evergreen Euro-Mediterranean members (*Q. coccifera* L., *Q. ilex* L.), with which *E. permagnificus* and *P. roseoalbidus* are also known to occur often in the Mediterranean (Galli 1998, Lannoy & Estadès 2001, Ladurner & Simonini 2003, Muñoz 2005). However apparently the phylogenetic relationship of the native hosts and the Red Oak does not provide a barrier in this case and a typical ‘novel association’ as termed by Dickie & al. (2010) has evolved. Similar new associations with non-native trees were thoroughly documented for some European species of *Suillus* Gray, involving host alteration between the two subgenera of *Pinus* L. (Moreau & al. 2015b). Such novel associations were recently shown to play an important role in other invasive tree species (Moeller & al. 2015). Moreau & al. (2015b) suggested that peculiarity of urban environment might somehow facilitate host alteration in mycorrhizal fungi. No members of the genera *Exsudoporus* and *Pulchroboletus* are reported so far as mycorrhizal with *Q. rubra*, although the species of those clades are known to occur in North America and other bolete genera were found in association with the Red Oak. As evident from Table 2, *Q. rubra* in America is a prolific host, related to a number of boletes from phylogenetically distant entities (cf. Wu & al. 2014). At least some European boletes appear to be versatile in their mycorrhizal association, as several species are known to enter association with the Australian *Eucalyptus* species (*Myrtaceae* Juss.) on the Iberian Peninsula: *Boletus edulis*, *B. reticulatus*, *Chalciporus piperatus*, *Imleria badia*, *Neoboletus erythropus* (Pers. : Fr.) C. Hahn, *Xerocomellus chrysenteron* [s. l.], and *Xerocomus subtomentosus* (Alvarez 2008). The concordance between the species of tubulate boletes, occurring in Europe with *Q. rubra* (Table 2), and

**Table 2.** Mycorrhizal boletes with tubulate hymenophore reported with *Quercus rubra*.

Current placement	Reported as	America	Europe	Reference
<i>Aureoboletus</i> s.l.	<i>Boletus russellii</i>	+		Homola & Mistretta (1977)
<i>Baorangia</i>	<i>Boletus bicolor</i> var. <i>bicolor</i>	+		Homola & Mistretta (1977)
<i>Boletellus</i>	<i>Boletellus chrysenteroides</i>	+		Homola & Mistretta (1977)
<i>Boletus</i> s. str.	<i>Boletus edulis</i> (1) <i>B. reticulatus</i> (2) <i>B. nobilissimus</i> (3)	(3)	(1, 2)	(1) Trocha & al. (2012)*, Meotto & Pellegrino (1989)** (2) Münzmay (1999), Assyov (2004), Sammler (2004), Scholler & al. (2014) (3) Dentinger & al. (2010) *
<i>Butyriboletus</i>	<i>Boletus roseopurpureus</i> (1) <i>Boletus appendiculatus</i> (2)	(1)	(2)	(1) Bessette & al. (2000) (2) Fernández & al. (2006)
<i>Chalciporus</i>	<i>Chalciporus piperatus</i>		+	Scholler & al. (2014)
<i>Hortiboletus</i>	<i>Xerocomus rubellus</i> [s.l.]		+	Münzmay (1999)
<i>Imleria</i>	<i>Xerocomus badius</i>		+	Sammler (2004)
<i>Neoboletus</i>	<i>Boletus erythropus</i> [aff.]	+		Walker & al. (2005)***
<i>Paragyrodon</i>	<i>Paragyrodon sphaerosporus</i>	+		Vellinga & al. (2006)
<i>Retiboletus</i>	<i>Boletus griseus</i>	+		Homola & Mistretta (1977)
<i>Tylopilus</i>	<i>Tylopilus felleus</i> (1) <i>Tylopilus rubrobrunneus</i> (2)	(2)	(1)	(1) Sammler (2004) (2) Homola & Mistretta (1977)
<i>Strobilomyces</i>	<i>Strobilomyces floccopus</i>	+		Homola & Mistretta (1977)
<i>Suillellus</i>	<i>Boletus luridus</i> [s.l.]		+	Münzmay (1999)
<i>Xerocomellus</i>	<i>Xerocomus chrysenteron</i> [s.l.]		+	Münzmay (1999), Sammler (2004)
Yet unresolved	<i>Boletus roodyi</i>	+		Ortiz-Santana & al. (2009)
Yet unresolved, possibly related to <i>Leccinellum</i>	<i>Leccinum rugosiceps</i>	+		Homola & Mistretta (1977)

Numbers in parenthesis in the last three columns correspond to those used in the second column. Remarks after literature sources: \* – identity of the fungus assessed by means of molecular techniques, \*\* – synthetic mycorrhization, \*\*\* – backed up with sequence, matching *Neoboletus* sequences, species unknown and therefore marked as “[aff.]”. Species in the second column, marked as “[s. l.]”, belong to difficult groups, in which additional species were recognized further after the date of the respective publication.

*Eucalyptus* spp. is easy to see. A fact worthy of attention is that the species of the above list sharing association with *Q. rubra* and *Eucalyptus* spp. are widespread in Europe. Some of them, as *B. edulis* and *I. badia* have been recorded with different host trees, both *Fagaceae* and *Pinaceae* (Ladurner & Simonini 2003, Muñoz 2005), although sometimes showing different preferences in some areas of their European range. In stark contrast, *E. permagnificus* and *P. roseoalbidus* are species with apparently narrower ecological requirements and concordantly limited geographic range. The versatility of all above-mentioned boletes towards the host-trees could not be reasonably explained at present, but certainly merits further attention.

## Conclusions

There is not much information about the fungal communities of *Q. rubra* in Europe, except probably about the wood-destroying species, which affect its cultivation. This paper documents an unexpected association between the Northern Red Oak and two

bolete species with very limited southern distribution. However, evidently information is not sufficient about the interactions of this non-native for Europe species and the indigenous ectomycorrhizal fungi. This calls for further research both above and below ground, considering the fact that this tree is reproducing successfully under certain European climates and is viewed as potential or proven invasive species in the different countries. As it is a tree of undoubted value in forestry and landscaping, a deeper understanding of adaptation of *Q. rubra* to local ectomycorrhizal species could also contribute to the practice of its cultivation in an environmentally safe manner.

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