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Dedicated to Professor Barbara Gumińska on the occasion of her eighty-fifth birthday

The first locality of *Chalciporus rubinus* (Boletales, Basidiomycota) in Poland

MAREK HALAMA and JERZY SZYPUŁA

Museum of Natural History, Wrocław University
Sienkiewicza 21, PL-50-335 Wrocław, marhalam@biol.uni.wroc.pl

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Chalciporus rubinus (W.G. Sm.) Singer, described in 1868 from England, was found in a city park in Wrocław. This is the first record of the species from Poland. Macro- and micromorphological characters of the Polish specimens are described and illustrated. The delimitation of *Ch. rubinus*, the knowledge of its distribution, ecology and conservation status is summarised.

Key words: *Chalciporus, Rubinoboletus*, urban greenery, Wrocław, SW Poland

INTRODUCTION

The genus *Chalciporus* Bataille belongs to *Boletaceae,* Boletales, Agaricomycetidae, Agaricomycetes, Basidiomycota, Dikarya, Fungi (Šutara 2005; Hibbett et al. 2007). It includes mycorrhizal, but usually not strictly specialized and not always obligatorily symbiotic fungi, occurring in temperate and tropical zones of both hemispheres. There are twenty three undoubtedly recognized *Chalciporus* species worldwide and three of them occur in Europe (Klofac, Krisai-Greilhuber 2006; Degreef, De Kesel 2008; Kirk et al. 2008; cf. Singer 1986; Horak 2005; Muñoz 2005; Šutara 2005). Only one species of the genus, *Ch. piperatus* (Bull.: Fr.) Bataille (Wojewoda 2003) has been reported from Poland so far.

The junior author found some fruiting bodies of an unusually coloured bolete species in the Szczytnicki Park, in the south-eastern part of Wrocław (SW Poland). The fungus was identified as *Chalciporus rubinus* (W.G. Sm.) Singer, a species not yet known from Poland. The aim of the paper is to describe the first collections of the species for Poland, and to summarise the current knowledge of its taxonomy, distribution, ecology and conservation status.

MATERIAL AND METHODS

Material was collected on one locality. The description of macroscopic features is based on fresh material, on seven collections, comprising more than 40 basidiomata in all stages of development. The microcharacters of three recorded basidiomata (from three collections: nr 1, 2, 6) were observed and measured under a light microscope at magnification 1500× (basidiospores) and 800× (other features). For microscopic observations, dried pieces of basidiomata were placed in 5% NH_4OH for about 5 minutes, then transferred to deionised water until they become pliable. Free-hand sections of the rehydrated pieces of basidiomata were examined in 5%NH_4OH, Congo Red and phloxine (in 1%NH_4OH). Amyloidity was tested with the Melzer's reagent. Morphological measurements were made and are presented according to the method presented by Breitenbach and Kränzlin (1991). The abbreviation *Q* is the ratio of basidiospore length to its width. Terminology of morphological and anatomical elements has been adopted mainly from Vellinga (1988). Reported size of basidiospores, basidia (with sterigmata) and cystidia (cheilocystidia, pleurocystidia, caulocystidia), as well as dimensions of pileipellis hyphae were based on 31, 21, 31 and 31 measurements, respectively. Basidiospore measurements, *Q* coefficient and cystidia are presented as the mean, standard deviation, with the minimum and maximum dimensions in parentheses. Dimensions of basidia are given as the range of minimum and maximum dimensions. Drawings were made with the aid of a drawing tube under an oil-immersion objective. The voucher specimens of *Ch. rubinus* have been deposited in the Herbarium of the Museum of Natural History, Wrocław University in Wrocław, Poland (WRSL).

RESULTS

Chalciporus rubinus was found for the first time in Poland on 12th of June 2007 in the Szczytnicki Park, in the south-eastern central part of Wrocław (Fig. 1). Five carpophores growing on the ground in the neighbourhood of *Tilia* and *Quercus* were observed at the time. During the following forays made on 13th, 19th and 29th of June 2007 a few dozen of carpophores were discovered at the same locality and its nearest surroundings. On these occasions *Ch. rubinus* was found under *Fagus*, *Fraxinus*, *Quercus*, as well as *Philadelphus*. Later in August and September of the same year, the species was found at the same locality again. Further observations confirming the occurrence of *Ch. rubinus* at the investigated site were conducted in the period between July and September 2008. The location of the species has been carefully marked and will be monitored in future.

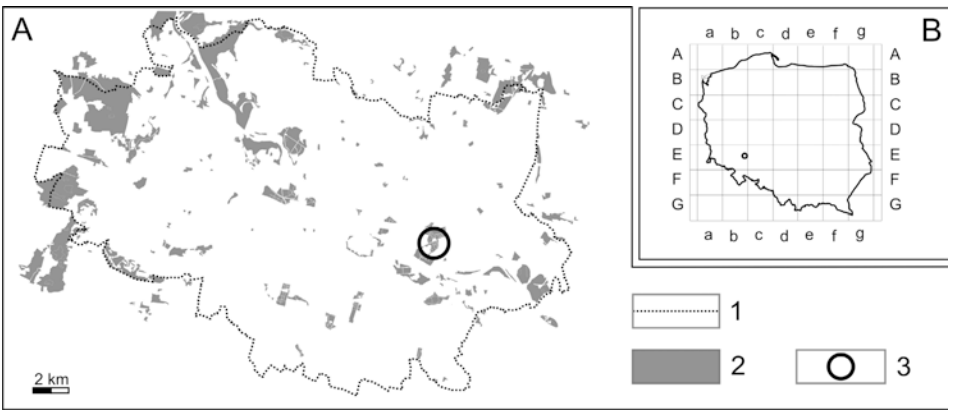

Fig. 1. The location of the *Chalciporus rubinus* site in Wrocław (A) and Poland (B; based on a 100 km ATPOL grid); 1 – urban boundary, 2 – municipal forests and parks, 3 – locality of the species.

DESCRIPTION OF THE SPECIMENS

Chalciporus rubinus (W.G. Sm.) Singer Figs 2–3

Persoonia 7 (2): 319. 1973, (syn.: *Rubinoboletus rubinus* (W.G. Sm.) Pilát & Dermek, *Suillus rubinus* (W.G. Sm.)Kuntze, *Xerocomus rubinus* (W.G. Sm.) A. Pearson).

Macroscopic and microscopic characters. Pileus 14-85 mm in diameter, hemispherical, subumbonate then plano-convex to even applanate and with reflexed margin; surface tomentose to finely fibrillose, dry and mat at first, lubricated and bright during wet weather, felted with age and even a little cracked during dry weather; brown, buff or alutaceous, usually keeping a pink tinge in about 5 mm from the margin. Tubes first adnate, then slightly decurrent, up to 6 mm long, first pink or locally light yellow, then crimson-red to pink, the colour not changing in contact with air. Pores irregular, angular, up to 1 mm in diameter, concolorous with the tubes, progressively becoming red-rose from the margin to the centre on age, the colour not changing under pressure. Stipe 15-50×5-20 mm, solid, cylindrical with a tapering base or cylindrical, often slightly curved, smooth or somewhat ribbed by the decurrent tubes on the top, above concolorous with the pores, below red-rose to yellow, usually with chrome-yellow basal mycelium. Context in pileus and stipe quite compact, slightly watery in pileus, fibrillous in stipe, in pileus whitish with reddish patches, in stipe whitish to yellow and warm yellow at the base. Smell indistinct or barely noticeable. Taste mild. Spore print pale brown. Basidiospores (4.1) 6.3 ± 0.7 (7.2) × (3.8) 4.3 ± 0.5 (6.4) μm, Q = (0.68) 1.47 ± 0.22 (1.76), ellipsoid to oblong, with a moderate hilar appendage, weakly pigmented, pale yellow (in 5%NH_4OH), with a large guttula, inamyloid. Basidia 30.4-43.3 × 9.3-11.7 μm, narrowly clavate, hyaline or containing small granules while immature, mostly with 4 sterigmata, without a basal clamp. Cystidia (cheilocystidia, pleurocystidia) (36.6) 47.1 ± 5.9 (58.2) × (4.9) 7.1 ± 1.6 (9.8) μm, narrowly

cylindrical to narrowly fusiform, potbellied, rarely narrowly clavate, erected or slightly curved, sometimes flexuose, dirty yellow (in 5%NH_4OH), somewhat encrusted with crystalline bodies. Caulocystidia (10.1) 27.0 ± 12.1 (53.5) × (2.2) 4.1 ± 1.4 (6.9) μm, cylindrical to clavate, usually articulate, mostly with obtuse apex, dirty yellow (in 5% NH_4OH), accompanied by some scattered caulobasidia. Pileipellis: a trichoderm transiting into a cutis, made up of usually interwoven, septate, cylindrical and thin-walled hyphae without clamps and with rounded, usually slightly broader (narrowly clavate to clavate) terminal elements (5,1-12) μm wide, with scattered incrustations and yellow-brown intracellular pigment (Figs 2, 3).

MATERIAL EXAMINED. 1. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Tilia* sp., *Quercus* sp., 12.06.2007, leg. J. Szypuła, WRSL; 2. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Fagus sylvatica*, *Fraxinus* sp., *Quercus* sp., *Philadelphus* sp., 13.06.2007, leg. M. Halama, WRSL; 3. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Fagus* sp., *Quercus* sp., 19.06.2007, leg. J. Szypuła, WRSL; 4. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Fagus* sp., *Quercus* sp., 29.06.2007, leg. J. Szypuła, WRSL; 5. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Fagus* sp., *Quercus* sp., 29.08.2007, leg. J. Szypuła, WRSL; 6. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Quercus* sp. div., 21.07.2008, leg. M. Halama, WRSL; 7. Wrocław (Poland), Szczytnicki Park, soil humus, in the neighbourhood of *Quercus* sp., 20.09.2008, leg. J. Szypuła, WRSL.

TAXONOMICAL REMARKS. In September 1866, near Dunstable (South Bedfordshire, Britain), Worthington G. Smith found plentiful fruiting bodies of previously unknown hymenomycetous fungus. It was clear to him, that observed basidiomata

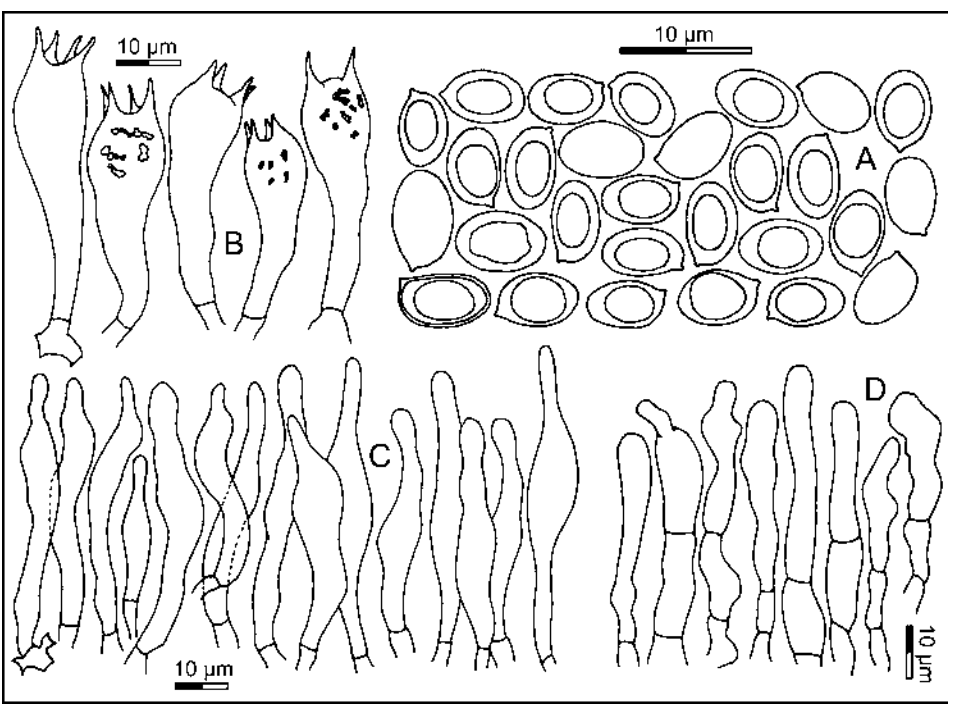

Fig. 2. Spores (A), basidia (B), cystidia (C) and caulocystidia (D) of *Chalciporus rubinus* recorded in Wrocław (12.06.2007, coll. by J. Szypuła; drawn by M. Halama).

Fig. 3. Fruit-bodies of *Chalciporus rubinus* recorded in Wrocław (Phot. M. Halama, 21.07.2008).

of the species belonged to the Group II, *Subtomentosi*, of Fries (1836-1838), and he described and illustrated the fungus under the name *Boletus rubinus* (Smith 1868). With improved knowledge of microcharacters and their application as generic criteria, the species have been placed in *Suillus* Gray (Kuntze 1893-1898; Singer 1965), *Xerocomus* Quél. (Skirgiełło 1960), *Rubinoboletus* Pilát & Dermek (Pilát, Dermek 1969) and *Chalciporus* Bataille (Moser 1983; Allesio 1985; Singer 1986; Horak 2005; Muñoz 2005; Klofac, Krisai-Greilhuber 2006). The approach of Pilát and Dermek (1969) was adopted by some authors of the most recent mycological papers (e.g., Šutara 2005; Knudsen, Taylor 2008; Šutara 2008). According to Pilát and Dermek (1969), the position of *Boletus rubinus* W.G. Sm. in such genera as *Xerocomus*, *Suillus* and *Chalciporus* was isolated; the common feature of these genera is the fact that all their species have a uniform, elongate boletoid shape of spores. Therefore, the transfer of this species to an independent genus, *Rubinoboletus*, seemed to be a fairly acceptable solution (Šutara 2005).

Nevertheless, the autonomous generic status of *Rubinoboletus* is still controversial. Šutara (2005) came to a conclusion that, with regards to the anatomical structure of the carpophores, *Chalciporus* is very similar to *Rubinoboletus*. He additionally found that it was very difficult to specify the boundary between the genus *Chalciporus* and *Boletus* subg. *Xerocomus* (Quél.) Maubl. He gave the spore print colour as the main but rather doubtful criterion to distinguish between European representatives of *Chalciporus* (cinnamon-brown or ferruginous-brown) and *Xerocomus* (brownish with more or less strong olive tinges), and separated *Rubinoboletus* on the basis of its short spores.

The smaller spore size was not regarded as an important feature at the generic level by Singer (1986). Degreef and De Kesel (2008) made a discovery of an interesting representative of *Chalciporus* in Africa. They described the species under the name *Chalciporus africanus* J. Degreef & De Kesel and reported that it was similar to the temperate *Ch. rubinus* (W.G. Sm.) Singer, but differed in its larger and more elongated spores, unchanging context and prominent reddish pileus colour. As a consequence, Degreef and De Kesel (2008) treated *Ch. africanus* as taxon bridging the generic difference between *Chalciporus* and *Rubinoboletus* Pilát & Dermek. In their opinion the close relationship between *Ch. africanus* and *Ch. rubinus* confirms Singer's (1986) opinion that *Boletus rubinus* W.G. Sm. is undoubtedly a good species of *Chalciporus*, making *Rubinoboletus* a synonym of *Chalciporus*. Degreef and De Kesel (2008) maintained that all globose-spored taxa subsequently combined in, or described under *Rubinoboletus* should be placed elsewhere. Moreover, they supported the Corner's (Corner 1972 after, Singer 1986) statement that "subglobose spores are to be expected in any alliance of elongated spores". Klofac and Krisai-Greilhuber (2006) took a similar approach; they proposed to include the genus *Rubinoboletus* as a sub-genus in *Chalciporus.* This point of view seems to be the most reasonable taxonomical concept and is also kept here.

Besides *Ch. rubinus*, two other species of the genus occur in Europe: *Ch. piperatus* (Bull.: Fr.) Bataille (syn. *Ch. hypochryseus* (Šutara) Courtec)) and *Ch. amarellus* (Quél.) Bataille (syn. *Ch. pseudorubinus* (Thirring) Pilát & Dermek; *Ch. pierrhuguesii* (Boud.) Bataille) (Klofac, Krisai-Greilhuber 2006). For identification, the basidiospores, the taste of flesh, the colour of tubes and pores are the most important

features. *Ch. rubinus* differs from other species of the genus mainly in the smaller size and broad elliptical to ovoid-spherical shape of basidiospores (Q < 2). Other significant features are not peppery (or even bitter) context, red-pink (reddish-ochraceous in age) pileus, carmine-red tubes and pores, red or carmine-red stipe with chrome-yellow base and occurring under deciduous trees (Muñoz 2005). Mild taste is also a specific feature of *Ch. amarellus*, while the distinct peppery taste is a distinctive character of *Ch. piperatus*. Fruiting bodies of *Ch. amarellus* are characterized by cream to pale orange-brownish or yellow-brownish (and sometimes pinkish-cream along the margin) pilei, pale yellow to intense yellow stipes and pink or reddish-pink (ochre with age) tubes and pores. The species is associated with *Abies, Picea* or *Pinus* and has a tendency to occur at high altitudes in the mountains (Gminder 1994; Horak 2005; Muñoz 2005). *Ch. piperatus*, besides its distinct peppery taste, is characterized by dark red to rusty-brown pores, rusty-yellow to reddish-ochre pileus and concolorous, but normally lighter, bright yellow or chrome-yellow towards the base stipe (Horak 2005; Muñoz 2005). The species seems to be associated with coniferous (*Pinus, Picea, Abies*) and deciduous trees (*Quercus, Fagus, Betula, Castanea*), but its mycorrhizal status is regarded as doubtful (Högberg et al. 1996; Knudsen, Taylor 2008). Some authors suspected *Ch. piperatus* of an association with *Amanita muscaria* (L.: Fr.) Lam. (Spooner, Roberts 2005). The relation of these species was confirmed by Veerkamp and Arnolds (2008), although the mechanism still has to be explained.

Habitat and distribution. Carpophores of *Ch. rubinus* appear in the summer and autumn (Skirgiełło 1960; Dermek, Pilát 1991). The species is considered to be a mycorrhizal fungus (Antonín et al. 2006; Knudsen, Taylor 2008), associated exclusively with deciduous trees (Singer 1965). It was usually observed under *Quercus* (Singer 1965; Allesio 1985; Dermek, Pilát 1991; Hardtke, Otto 1999; Horak 2005; Legon et al. 2005; Muñoz 2005; Antonín et al. 2006; Arnolds, Veerkamp 2008; Knudsen, Taylor 2008), but also the records from the neighbourhood of *Fagus*, *Tilia*, *Castanea, Ilex* and *Crataegus* are known (Michael, Hennig 1971; Muñoz 2005; B.M.S. 2009). Everywhere in Europe, *Ch. rubinus* was generally recorded under isolated trees in old parks on alluvial riverside habitats (Legon et al. 2005; Kreisel 2006; Arnolds, Veerkamp 2008). Moreover, it was also found in warmer deciduous and mixed forests, in roadside verges planted with trees, in gardens and on playing fields (Skirgiełło 1960; Antonín et al. 2006; B.M.S. 2009).

Ch. rubinus is known hitherto only from Europe. In the 1960s it was considered to be a very rare taxon, known only from England, former Czechoslovakia and Germany (Saxony) (Singer 1965). Nowadays the species is widespread but regionally usually treated as very rare (Muñoz 2005; Arnolds, Veerkamp 2008). The present distribution area of *Ch. rubinus* extends mainly throughout the West to Central Europe, but localities scattered in southern and northern parts of the continent are also known. It is known from England (Legon et al. 2005), Germany (Kreisel 1987; Kleine et al. 2004), the Czech Republic (Polčák 2003; Skála 2003; Antonín et al. 2006), the Netherlands (Keizer 1995; Beenen et al. 2002), Belgium (Van de Kerckhove 2001; Van de Kerckhove, Walleyn 2006), Bulgaria (Assyov, Denchev 2004), Slovakia (Lizoň 2001), Austria, Hungary (Allesio 1985; Muñoz 2005) as well as Italy (Allesio 1985), Spain (Rubio et al. 2006) and Norway (Bendiksen et al. 1999).

Recently some authors have drawn mycologists' attention to changes in the distribution of *Ch. rubinus*. Antonín et al. (2006) has reported the species as an example of spreading taxon, gradually increasing its previous limited distribution range in the Czech Republic. This tendency has also been confirmed by Kreisel (2006) in Germany. Nevertheless, earlier recognized as a rare species, *Ch. rubinus* was included in the red lists of the above and other European countries. In the Czech Republic, Germany and Bulgaria it is treated as an endangered species (Benkert et al. 1992; Gyosheva et al. 2000; Lizoň 2001), in Great Britain, Norway and Slovakia it is regarded as vulnerable (Bendiksen et al. 1999; Lizoň 2001; Legon et al. 2005), while in the Netherlands as near threatened (Arnolds, Veerkamp 2008).

For many years fungus forays have remained concerned with potentially rich habitats in order to record the widest range of species diversity. As a result, they focused on rural sites, leaving urban areas with only occasional recording. In order to find a new localities of *Ch. rubinus* it is necessary to investigate man-made landscape. Anthropogenic habitats may usually appear worthless for fungi, but actually they present a variety of challenges for studies.

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Pierwsze w Polsce stanowisko *Chalciporus rubinus* (Boletales, Basidiomycota)

Streszczenie

Autorzy prezentują pierwsze w Polsce stanowisko maślaczka rubinowego – *Chalciporus rubinus*. Owocniki tego gatunku, rosnące na ziemi, w sąsiedztwie różnych drzew liściastych (głównie dębów), zostały znalezione po raz pierwszy dnia 12 lipca 2007 roku w Parku Szczytnickim we Wrocławiu. W pracy przedstawiono charakterystykę oraz zilustrowano najważniejsze cechy budowy makroskopowej i mikroskopowej znalezionych owocników, a także przybliżono taksonomię, ekologię i europejskie rozmieszczenie odnotowanego gatunku.