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ORIGINAL RESEARCH PAPER

Rubroboletus le-galiae (Boletales, Basidiomycota), a species new for Poland

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Abstract

Rubroboletus le-galiae is reported for the first time from Poland. Macro- and micromorphological characters of the species are described and illustrated based on the study of material collected at three microlocalities in Łężczok reserve (SW Poland). The delimitation of *R. le-galiae* from related species of the genus *Rubroboletus* (*R. satans*, *R. rubrosanguineus*, *R. rhodoxanthus*) is shortly discussed and the knowledge of its ecology and distribution is briefly summarized.

Keywords

Boletus legaliae; *Boletus spinarii*; *Rubroboletus*; *Suillellus*; ectomycorrhizal fungi; Poland

This paper is dedicated to Professor Maria Lisiewska and Professor Anna Bujakiewicz on the occasion of their 80th and 75th birthday, respectively.

Introduction

Modern molecular techniques have revolutionized the study of fungal taxonomy, systematics, phylogeny and biogeography (e.g., [1–3]). With these approaches, it was found that genus *Boletus* as it has been understood so far (e.g., [4–9]) was an artificial grouping (e.g., [10–17]). Although, *Boletus* sect. *Luridi* Fr. sensu Singer [18] is well-defined group, distinguished macromorphologically in the boletoid habit, the small, often orange-reddish pores, the reticulate or finely furfuraceous stipe surface and usually blueing tubes, pores and context, it has proven to be polyphyletic [19–20]. Based on molecular data and morphological features, the genus *Suillellus* was recently reconfirmed and a batch of new genera of boletes were erected (*Exsudoporus*, *Imperator*, *Neoboletus*, *Rubroboletus*) to accommodate various members of the sect. *Luridi* [21–30]. The section *Luridi* of the genus *Boletus* s. l. comprises 20–25 species in Europe [4,31–33] and so far six of them have been reported to occur in Poland: *Neoboletus luridiformis* (Rostk.) Gelardi, Simonini & Vizzini (syn. *Boletus luridiformis* Rostk), *Neoboletus junquilleus* (Quél.) Gelardi, Simonini & Vizzini (syn. *B. luridiformis* Rostk. var. *junquilleus*), *Suillellus luridus* (Schaeff.: Fr.) Murrill (syn. *Boletus luridus* Schaeff.: Fr.), *Suillellus queletii* (Schulzer) Vizzini, Simonini & Gelardi (syn. *Boletus queletii* Schulzer), *Rubroboletus rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang [syn. *Boletus rhodoxanthus* (Krombh.) Kallenb.], *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang (syn. *Boletus satanas* Lenz) ([34] and subsequent literature therein). In August 2014, during a mycological survey of the Łężczok reserve (SW Poland), one rare bolete of this section, namely *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli (syn. *Boletus le-galiae* Pilát & Dermek) was found for the first time in this country (Fig. 1). The aim of this paper is to describe the first collections of *R. le-galiae* for Poland, to compare their characters with published data and to summarize briefly current knowledge of its taxonomy, ecology and distribution.

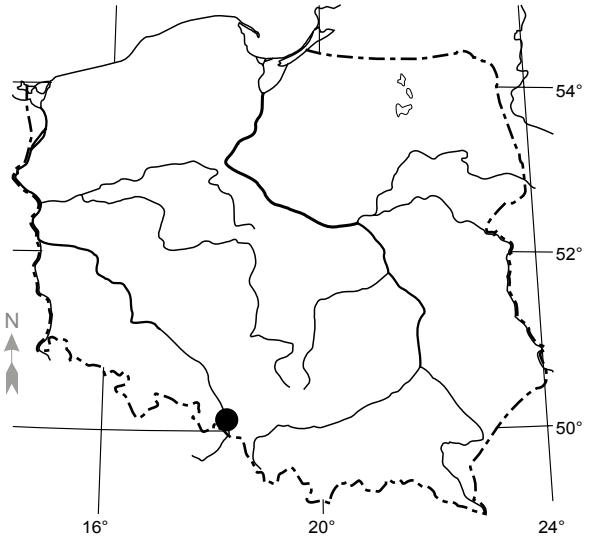

Fig. 1 Known distribution of *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli in Poland (black circle).

Material and methods

Specimens were collected, documented and preserved using standard methods. Macroscopic features were studied from fresh material of four collections comprising six-basidiomata in different stages of development growing at three microlocalities. All microscopic structures were observed in dried material. Freehand sections of rehydrated pieces of basidiomata were examined in 5% $NH_3 \cdot H_2O$ and Congo Red reagent. Squash slides for observation of basidia and cystidia were made from sections of the hymenophore. Sections of pileipellis were prepared as suggested by Ladurner and Simonini [35]. Spore preparations were made by submerging small pieces of hymenophore in mounting medium for approximately 30 s to release the spores. Microcharacters were observed with a Nikon Eclipse E-400 light microscope equipped with a Nikon digital camera (DS-Fi1). Image-grabbing and biometric analyses were done with NIS-Elements D 3.1 imaging software. Dimensions of microcharacters are given as (minimum) average ± standard deviation (maximum), and additionally in the form of the main data range (10–90 percentile values). The *Q* value is the length/width ratio of basidiospores. For basidiospores size measurements, randomly selected mature spores were measured. The length of basidia was measured excluding sterigmata. Microphotographs were taken using digital camera. Statistical computations employed Statistica software (StatSoft). The species concept follows Muñoz [4] and the morphological terminology follows Ladurner and Simonini [35]. The studied collections are deposited in the Museum of Natural History, Wrocław University, Wrocław, Poland (WRSL).

Results

Rubroboletus le-galiae (Pilát & Dermek) Della Maggiora & Trassinelli
Index Fungorum 246: 1. 2015 ≡ *Boletus le-galiae* Pilát & Dermek, Houby Československa ve svém životním prostředí (Praha): 52. 1969 (for complete synonymy see Index Fungorum; for further details on the nomenclature of the species the reader is referred to the thorough discussion presented by Redeuilh [36] and Mikšík et al. [37]).

Illustrations. Muñoz [4]: 745–747, photo 69a–69f; Šutara et al. [38]: 181–183, photos; Breitenbach and Kränzlin [39]: 65, fig. & photo 23 (as *B. splendidus* ssp. *splendidus*); Marchand [40]: 41, photo (as *B. satanoides*); Rald [41]: 8, photo; Vesterholt and Holm [42]: 8, photo; Engel et al. [43]: 135, plate 36 (as *B. splendidus*); Anderson [44]: 11–12, photos; Courtecuisse and Duhem [45]: 437, fig. 1699; Phillips [46]: 280, photo b; Pilát and Ušák [47]: plate 4 (as *B. purpureus* var. *le-galiae*).

Basidiomata growing solitary or gregarious. Pileus 40–170 mm in diameter and 15–30 mm in high, initially hemispherical, than convex to plano-convex, at first almost white to greyish white, soon becoming flushed with pinkish (especially at the cap margin), later entirely dingy pink to dark pink or beige with irregular pink spots, initially finely velvety, then smooth (and glistening), blueing only after rough handling. Tubes pale yellow to yellow, blueing when injured, 1.3–7 mm in high. Pores small, initially yellow to golden yellow or golden yellow and flushed with orange, quickly becoming orange-red or remaining yellow and then with weak orange flush near the stipe, blueing when bruised. Stipe 40–100 × 20–90 mm, generally ± swollen to bulbous or club-shaped towards base, in the uppermost part bright to dark yellow, downwards orange red, pinkish orange to pinkish, with fine yellow (in the upper part of a stem), orange red to pinkish orange (in the lower part) network, clearly disappearing towards base, stipe

surface blueing when handled. Flesh whitish to very pale yellow, sometimes with reddish spots in the stipe base, blueing when exposed to air. Smell weakly fungussy when fresh and somewhat spicy and persistent (like "maggi" soup seasoning) when dried. Taste of fresh basidiocarp mild to slightly acid (Fig. 2a,b, Fig. 3a–c).

Basidiospores (10.4) 12.7 ±1.0 (16.4) × (5.4) 6.0 ±0.3 (7.0) µm, 11.6–14.0 × 5.7–6.5 µm, Q = (1.7) 2.1 ±0.2 (2.8), Q = 1.9–2.4 (n = 172), subfusiform in side-view, elongate ovoid to subfusiform in front view, smooth, thick-walled, yellowish in ammonia, ochre in Melzer's reagent. Basidia (24.7) 31.5 ±3.3 (39.2) × (9.0) 10.5 ±0.6 (12.1) µm, 27.0–35.2 × 9.7–11.2 µm (n = 52), clavate, 2–4-spored. Pleurocystidia (18.2) 26.3 ±6.2 (48.2) × (5.4) 7.1 ±0.9 (10.4) µm, 20.3–33.1 × 6.1–7.9 µm (n = 41), fusiform to lageniform, thin-walled. Cheilocystidia similar to the pleurocystidia. Caulocystidia (19.1) 29.3 ±6.8 (50.7) × (7.4) 10.2 ±1.5 (15.4) µm, 22.1–38.9 × 8.5–11.9 µm (n = 51), observed at the apex of stipe, abundant, similar to hymenial cystidia, but rather more polymorphic. Pileipellis an intricate trichoderm, ± collapsing with age, composed of cylindrical, interwoven septate hyphae, 3.3–7.8 µm wide, with typically obtuse, rarely swollen apices; hyphae smooth or with slightly incrusting pigment, some with brown contents. Clamp-connections not observed (Fig. 4a–d).

Fig. 2 Basidiomata of *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli (**a**,**b** – HM-2014-0281).

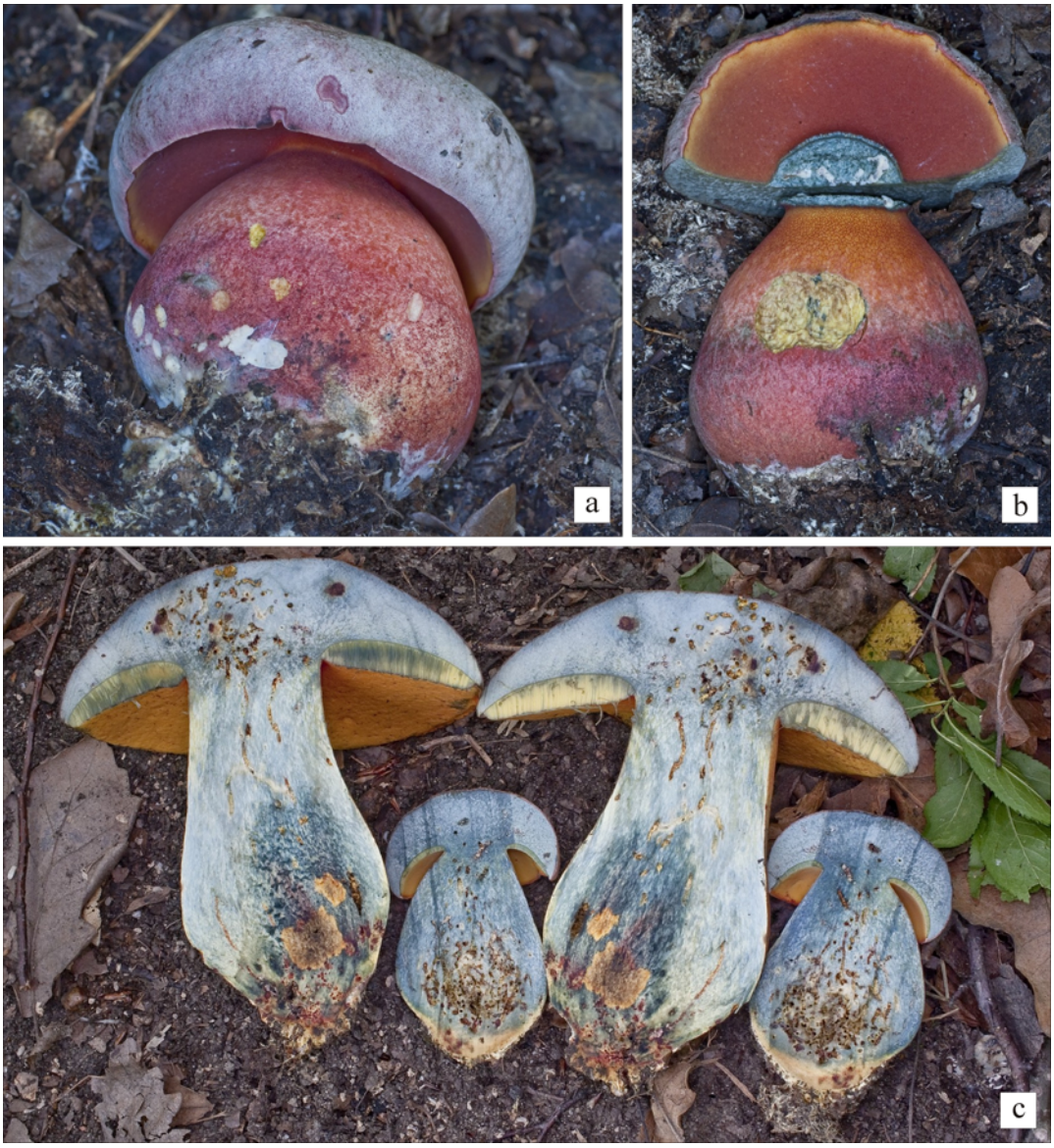

Fig. 3 Basidiomata of *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli (**a**,**b** – HM-2014-0278, **c** – HM-2014-0281).

Specimens examined. Poland, Silesian Lowlands, Kotlina Raciborska basin (Łężczok reserve), on dam of "Babiczak Północny" and "Salm Duży" ponds – on the Oak Alley: 1 – on soil (humus covered by broadleaved litter) under *Quercus* sp., *Carpinus betulus*, *Crataegus* sp., alt. 179 m a.s.l., 6 Aug. 2014, leg. M. Halama (WRSL: MH-2014-0278); 2 – on soil (humus covered by broadleaved litter) under *Quercus* sp., *Carpinus betulus*, *Crataegus* sp., *Sorbus aucuparia*, alt. 179 m a.s.l., 6 Aug. 2014, leg. M. Halama (WRSL: MH-2014-0279); 3 – on soil (humus covered by broadleaved litter) under *Quercus* sp., *Carpinus betulus*, *Crataegus* sp., alt. 179 m a.s.l., 6 Aug. 2014, leg. M. Halama (WRSL: MH-2014-0280, MH-2014-0281).

Additional collections examined. *Rubroboletus rubrosanguineus* – Slovakia, the Žilina Region, Dolný Kubín District, ca. 1.4 km SW from Demkovská, spruce forest, on soil (humus covered by coniferous litter) under *Picea abies*, *Sorbus aucuparia*, *Acer pseudoplatanus*, alt. 854 m a.s.l., 21 Aug. 2015, leg. R. Rutkowski, det. R. Rutkowski (WRSL: RRy-2015-0001); *Rubroboletus rhodoxanthus* – Slovakia, the Trenčín Region, Nové Mesto nad Váhom District, ca. 1.9 km SE from Nová Bošáca, beech forest, on soil (humus covered by broadleaved litter) under *Fagus sylvatica*, *Quercus* sp., alt. 333 m a.s.l., 9 Sep. 2015, leg. R. Rutkowski, det. R. Rutkowski (WRSL: RRy-2015-0002).

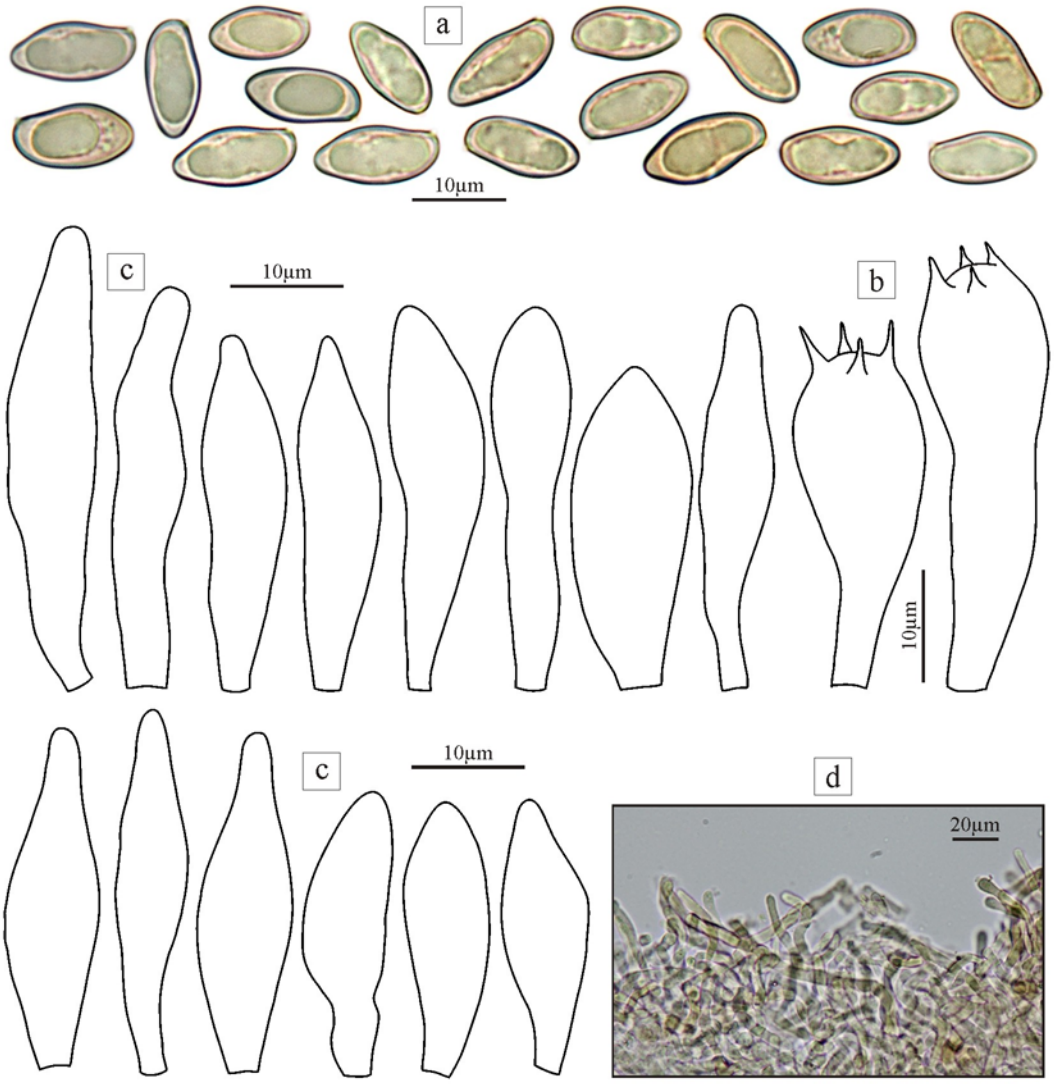

Fig. 4 *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli. **a** Basidiospores. **b** Basidia. **c** Pleurocystidia. **d** Pileipellis. All photographed and traced from HM-2014-0281.

Discussion

Morphological variability

Rubroboletus le-galiae is distinguished macroscopically by the colors of basidiomata, the evident reticulum which typically does not cover the entire stipe and is well developed on its upper half, as well as by its flesh changing when exposed to air. Another feature of this bolete is its dependence on the fulfillment of certain special requirements of soil, temperature and its symbiotic relationships.

Rubroboletus le-galiae is related to *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang, which occurs often in similar habitats, from which it differs in the presence of continuous pink-red layer beneath the pileipellis, different coloring of the mature pileus (with a pink tinge), typically the smaller basidiocarps and more slender stem, and its flesh, which, even when old, does not smell repulsively. *Rubroboletus le-galiae* can be also confused with *R. rubrosanguineus* (Cheype) Kuan Zhao & Zhu L. Yang., especially in young specimens. In *R. rubrosanguineus*, however, the pileus typically evolves more distinctive representation of purple pigments, and the blood red to purple coloring soon embraces the whole of its surface, and not becoming pink as in *R. le-galiae*. Moreover, the stipe of *R. rubrosanguineus* becoming immediately purple and blood red in color and is covered with fine and dense, concolourous network, while in *R. le-galiae* the stipe is orange-red, orange-yellow to yellow in the upper half and

orange red, pink-reddish to pinkish in the lower, and the reticulation seems wider, less crowded and is never purple or blood red colored. Finally, the habitats of these species are different: ecologically, *R. rubrosanguineus* is a typical species of the higher elevations and generally of the *Picea* and *Abies* mountain forests. Phenotypically similar relative is also *Rubroboletus rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang, however, which seems quite unmistakable for the color of the pileus (white, greyish-white to ochraceous then soon flushing pink with age) and stipe (reddish or yellow and covered with a crowded red network), and for the characteristic color change of the golden-yellow flesh, which typically becoming blue only in the pileus and remains unchanged in the stipe. Instead of the macrocharacters, the spore dimensions will most likely also prove useful features for microscopic separation of *R. le-galiae* from *R. rubrosanguineus* and *R. rhodoxanthus*. In particular, the width of the spores and ratio of length and width (*Q*) seems a good delimiting character, although spore variability is always present to a certain degree (Fig. 5, Fig. 6). Therefore, a statistically significant number of spores must be measured for a reliable separation of these species.

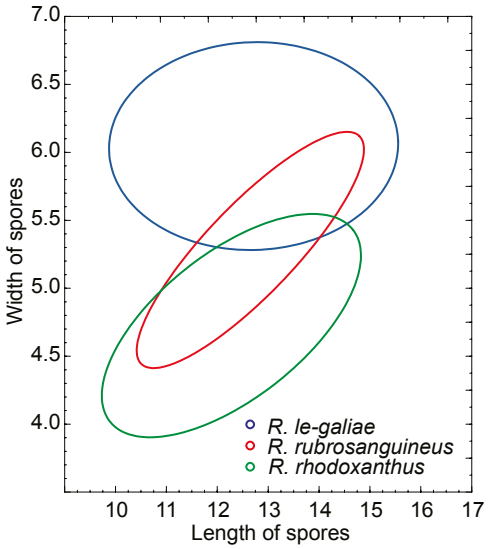

Fig. 5 Spore size of *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli (HM-2014-0281), *Rubroboletus rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang (RRy-2015-0002), and *Rubroboletus rubrosanguineus* (Cheype) Kuan Zhao & Zhu L. Yang (RRy-2015-0001). The lines are drawn on the basis of scatter diagrams and contain 95% of the spore measurements of each species.

Polish collections of *Rubroboletus le-galiae* correspond principally both macro- and microscopically to the descriptions available in the literature [4,5,7,38,48–50]. Nonetheless, the new findings presented here are characterized by a wide range of variability of colors of pores and stipes, what can be noticed in presented figures (Fig. 2a,b, Fig. 3a,b), showing the scope of color tints in different specimens at different stages of development. While *R. le-galiae* was originally described with "vividly scarlet to purple pores, which are fading away over time" [47], its form with yellow pores [viz. *Rubroboletus legaliae* f. *spinarii* (Hlaváček) Mikšík] is also known [38,51,52]. No constant differences could be observed for Polish collections of *R. le-galiae* with reference to the feature mentioned above. Starting from the innately pure yellow or scarlet color of the pores I have observed a gradual transition from orange red to almost yellow shades in old specimens. And the color variability of the stipe includes its upper half surface that in fresh basidiocarps was innately orange red, and then orange yellow to bright yellow. According to Šutara et al. [38] *R. le-galiae* shows significant variation in the pileus color that can vary considerably between different specimens due to their maturity and the influence of weather conditions. However, regarding Polish specimens this variability was not so significant and was only observed in relation to the stage of development. The odor of *R. le-galiae* has been described variously by authors as a strong and somewhat spicy [46,48], pleasant, of hay or chicory [4,50,53–55], subtle and fruity [56], and faint fungussy when fresh and like maggi soup seasoning when dried [38]. The last mentioned description of the feature seems to correspond best to my feeling about the flesh odor of this bolete.

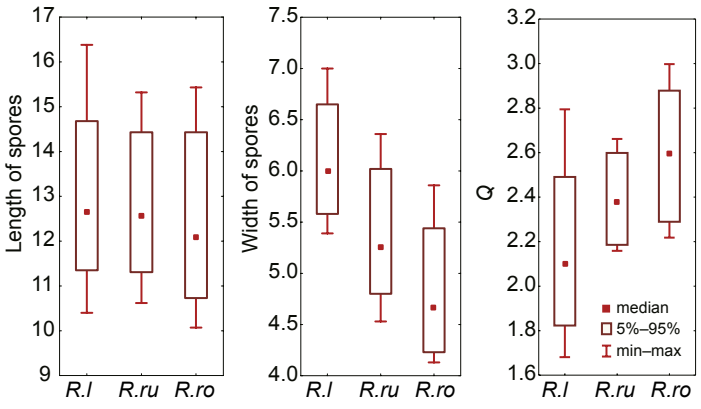

Fig. 6 Box plots for spore length, width and *Q* of *Rubroboletus le-galiae* (Pilát & Dermek) Della Maggiora & Trassinelli (*R.l*; HM-2014-0281), *Rubroboletus rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang (*R.ru*; RRy-2015-0002), and *Rubroboletus rubrosanguineus* (Cheype) Kuan Zhao & Zhu L. Yang (*R.ro*; RRy-2015-0001). Boxes represent the median, the upper and lower quartiles, and the extreme values.

Microscopically, the Polish collections demonstrate some differences in the spore size from the measurements reported by earlier authors. These discrepancies are probably attributable to the different sampling sizes: 172 basidiospores in this study, 142 basidiospores measured by Muñoz [4], and an unknown number by the other authors (e.g., [38,47,48,56]).

Distribution, habitat and fructification

Rubroboletus le-galiae, so far as known only in Europe, has a wide distribution in this continent, but it is nowhere regarded as common [7,38,49,54]. Apart from Poland, this bolete is known from Austria, Bulgaria, Croatia, Denmark, France (including Corsica), Germany, Great Britain, Hungary, Italy, Montenegro, Norway, Slovakia, Slovenia, Spain, Sweden, Switzerland, the Czech Republic, Romania, Serbia and Turkey (e.g., [4,44,45,49,57–62]). *Rubroboletus le-galiae* seems to be more frequent only in warmer regions of Central Europe [56] and in southern part of British Islands [48,50,63]. This species is recognized as very rare in Northern Europe [49] and is included in the red lists of endangered macrofungi in several countries, including Denmark, Great Britain, Norway, Slovakia, Sweden and the Czech Republic [62–67]. In general the records of *R. le-galiae* are from the lowlands, uplands, submontane and mountain areas with most finds up to about 800 m alt. The highest altitudes are from the Eastern Dolomites (up to 1100 m alt.) and Northern Apennines (up to 1400 m alt.) in Italy [54].

The most common habitats of *R. le-galiae* in South, Central, and Northwestern Europe are described by different authors as (natural, thermophilic or moderately moist and cool) open deciduous forests, wood pastures, parklands, fishpond dikes, wooded grasslands, and roadsides, typically in the sun-drenched locations (rarely in shaded places), on rich (acid, neutral or calcareous) soils, mainly with a dominant presence of *Quercus* (*Q. petrea*, *Q. robur*, *Q. pyrenaica*, more rarely *Q. ilex*), *Fagus* (*F. sylvatica*), and more rarely *Castanea* (*C. sativa*) and *Carpinus* (*C. betulus*) [4,7,38,44,47,49,54,63,65,68]. The Polish records of *R. le-galiae* are confined to three isolated but homogeneous, warm exposed microlocalities within a single fishpond dike in the Łężczok reserve. The minimum distance between separate microlocalities is ca. 50 m. At these sites *R. le-galiae* shows a prominent tendency to occur under *Quercus* and *Carpinus* on rich soil with a clayey and probably calcareous binding material. This coincides with the statements of Mikšik [69] who characterizes this species as growing mostly on fishpond dikes, and of Holec and Beran [65], Legon et al. [70], Kibby [50], and Ainsworth et al. [63] who indicate the oak trees as a main mycorrhizal hosts of *R. le-galiae* in the Czech Republic and in England. The observations of *R. le-galiae* from the Łężczok reserve correspond to the result of the investigation by Ainsworth et al. [63] who believe that this species is often found in bolete "hot spots". At the localities of *R. le-galiae* within the fishpond dike, several other interesting and rare mushrooms were observed; some of them are very characteristic of thermophilous habitats, including *Aureoboletus gentilis* (Quél.) Pouzar, *Rubinoboletus rubinus* (W.G. Sm.) Pilát & Dermek, *Caloboletus radicans* (Pers.: Fr.) Vizzini, *Boletus reticulatus* Schaeff., *Boletus aereus* Bull.: Fr., *Leccinellum crocipodium* (Letell.) Della Maggiora & Trassinelli, *Suillellus luridus* (Schaeff.: Fr.) Murrill, and also *Tricholoma sejunctum* (Sowerby: Fr.) Quél. and *Tricholoma ustaloides* Romagn. Since all of the above-mentioned localities of *R. le-galiae* are situated in a protected area the species seems to be locally nonthreatened.

According to Šutara et al. [38] basidiocarp production of *R. le-galiae* takes place from June to October. The annual field experience from the Łężczok reserve suggests that the first part of August is the most suitable period for basidiocarp production of the species. However, further observations are needed to recognize sufficiently the periodicity of *R. le-galiae* in the area. While there are insufficient field observations, the question of the possible fluctuations of the species at the recorded localities remains also an open one.

Practical significance

Opinions as to whether *Rubroboletus le-galiae* is edible, non-edible or poisonous vary widely in the literature [56,71–73]. Some authors argue that it is poisonous when raw or after an insufficient heat treatment [47,56,72]. Gry and Andersson [74] believe that *R. le-galiae* is poisonous and it gives gastrointestinal adverse symptoms. In general, the responsible toxicant(s) and toxic properties of "inedible" species of *Boletus* s. l. are insufficiently known [75–78], and those of *R. le-galiae* are apparently, as yet, unidentified [74].

Conclusions

Rubroboletus le-galiae is a striking but highly variable mushroom that is not always easily recognizable. In the case of the recorded specimens it demonstrates extremely variable coloring of pores and stipes. This bolete apparently belongs to extremely rare species in Poland. It will be interesting to see if further records of *R. le-galiae* can be found in different parts of the country. Using the knowledge of its habitat requirements, the broader distribution of the fungus can be expected here. *Rubroboletus legaliae* is of no practical importance as food, but it may become important source of new medicines in the future. From a biodiversity conservation perspective, I propose to add this bolete to the next edition of the red list of the macrofungi in Poland and classify it in the data deficient (DD) category. The main reason is that the general distribution of *R. le-galiae* in Poland is not satisfactorily known yet and this species seems to be well integrated into the thermophilous fungal communities of rare and very rare species. Further findings of *R. le-galiae* should be recorded and documented in the country. The author would welcome such reports.

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