

Article ID: 5529
DOI: 10.5586/am.5529

Publication History
Received: 2020-08-06
Accepted: 2020-03-11
Published: 2021-02-25

Handling Editor
Andrzej Szczepkowski; Warsaw
University of Life Sciences –
SGGW, Poland;
<https://orcid.org/0000-0002-9778-9567>

Authors' Contributions
PM: study concept, field research, species identification, preparation of manuscript, figures and table; MK: study concept, field research, species identification, preparation of distribution maps; FK: study concept, field research, editing and correcting of the final version of the manuscript



Funding
This study was financially supported by the Forest Fund of the State Forests National Forest Holding through the Tatra National Park, within the project "Diversity and distribution of hypogeous fungi in the Tatra National Park" (2019), and by the Polish National Science Centre within the project N N305 304540 "Hypogeous Basidiomycota in Poland against their occurrence in Central Europe."

Competing Interests
No competing interests have been declared.

Copyright Notice
© The Author(s) 2020. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.

CHECKLIST

New Records of Rare Hypogeous Fungi from Poland (Central Europe)

Piotr Mleczko ^{1*}, Maciej Kozak², Filip Karpowicz ¹

¹Institute of Botany, Faculty of Biology, Jagiellonian University, Kraków, Poland

²Independent researcher, Kraków, Poland

*To whom correspondence should be addressed. Email: piotr.mleczko@uj.edu.pl

Abstract

Investigations carried out in recent years have provided new data on the localities of some rare hypogeous fungi in Central Europe. In this study, we present new localities of *Leucangium carthusianum*, *Melanogaster luteus*, and *Rhizopogon melanogastroides*, two of which are new for Poland. Sporocarps of *L. carthusianum* were found in seven new localities in mixed and coniferous forests in the Western Carpathians as well as in Sudetes. In Poland, the species was also recorded in a mixed forest in the Kraków-Częstochowa Upland. Fir, *Abies alba*, accompanied the fungus in almost all known localities. Three localities of *M. luteus* were found in the Polish Western and Eastern Carpathians in *Alnus incana* communities mostly associated with streams. Knowledge of the distribution of this species in Europe is incomplete due to the complicated taxonomic history; nevertheless, it is regarded as rare, despite its wide distribution. One new locality of *R. melanogastroides* recorded in the Tatra Mts, Western Carpathians, is the fourth known to date. This species is mostly associated with *Pinus mugo* in high mountain localities (the Alps, the Tatra Mts). In this study, detailed descriptions and illustrations of the macro- and micromorphological features of the species are provided.

Keywords

macroscopic fungi; truffle-like fungi; hypogeous Ascomycota; hypogeous Basidiomycota; *Leucangium carthusianum*; *Rhizopogon melanogastroides*; *Melanogaster luteus*

1. Introduction

Hypogeous macrofungi are rarely considered in mycological investigations. Although considerable progress in knowledge of their taxonomy, diversity, and distribution has been made in recent years (e.g., Alvarado et al., 2016; Moreau et al., 2011; Paz et al., 2016, 2017; Stielow et al., 2011; Vidal et al., 2019), they remain one of the most understudied groups of fungi in Poland and other Central European countries.

Over 20 species of hypogeous Basidiomycota and nearly 30 species of hypogeous Ascomycota were found in Poland before the year 2000 (Chmiel, 2006; Ławrynowicz, 1988; Wojewoda, 2003). Since then, several studies have reported on the localities of new species hitherto not known to occur in Poland (e.g., Chachuła, 2018; Chachuła et al., 2020; Hilszczańska et al., 2008; Mleczko et al., 2009, 2010; Paz et al., 2017; Vidal et al., 2019; Wojewoda et al., 2016). Some recorded fungi are widespread and, at least locally, common; for example, *Elaphomyces asperulus*, *E. granulatus*, and *Octaviania mutabilis* (as *O. asterosperma*) (Ławrynowicz, 1988; Mleczko et al., 2009). The status of certain species regarded as very rare changed when more data on their distribution became available; for example, *Chamonixia caespitosa* (Kozak et al., 2013; Mleczko et al., 2010). However, several species have only been found in a single or only a few localities in

Poland, for example, *Elaphomyces aculeatus*, *Gautieria othii*, *Lactarius stephensii*, *Russula candidissima*, and *R. cerea* (Chachuła et al., 2020; Ślusarczyk, 2018; Vidal et al., 2019).

The aim of this study was to report on new localities of three rarely recorded species in Central Europe: *Leucangium carthusianum*, *Melanogaster luteus*, and *Rhizopogon melanogastroides*. Two of which, *M. luteus* and *R. melanogastroides*, are reported here for the first time in Poland.

2. Methods

Sporocarps of hypogeous fungi were found during field work carried out in southern Poland, in the Polish Carpathians in particular, in the years 2011–2019. The sporocarps were excavated by the careful removal of litter and outer soil layers to a depth of a few centimeters with the aid of rakes from an area of approximately 0.5 m². After exploration, the soil and litter were replaced in the excavation area.

The geographical coordinates of the localities were established using GPS Garmin 62s. Information regarding the type of plant community, associated ectomycorrhizal plant species, and elevation were noted. When possible, photos of the sporocarps were taken with a digital Nikon D90 camera with a Sigma 17–70 mm lens.

The identification of sporocarps was based on contemporary keys and monographic papers of Martín (1996), Montecchi and Sarasini (2000), and Moreau et al. (2011). Macroscopic features were observed on fresh and dry specimens. Microscopic features were observed using a ZEISS AxioScope A1 light microscope with DIC and digital camera (AxioCam MRc5) on handmade preparations of water-rehydrated sporocarps and were mounted in water, 3% KOH, and Melzer's reagent. The measurements of the microscopic structures necessary for sporocarp identification were performed using the computer image acquisition and analysis software ZEN 2012, blue edition (Carl Zeiss Microscopy). Collections of identified sporocarps are preserved in the Herbarium of the Institute of Botany, Jagiellonian University in Kraków (KRA), Poland.

Information regarding the locality, presented below, includes: (i) the square in the ATMOS grid (Wojewoda, 2000), (ii) the macro- and mesoregion, (iii) data of the locality (the name of the village, nature reserve, hill, mountain, valley, etc.), (iv) plant community type or plant association (if known), (v) associated ectomycorrhizal tree species, (vi) elevation, (vii) collection date, (viii) names of collecting and identifying persons, and (ix) herbarium specimen number. Where a locality is reported in a previous study, the source of information is quoted.

The notes present information on characteristic features, taxonomic position, ecology, and known distribution of species in Central Europe in particular. Information regarding synonyms is according to the Index Fungorum and MycoBank, unless specified otherwise.

3. Species and Localities

3.1. *Leucangium carthusianum* (Tul. & Tul.) Paol., Syll. fung. 8: 900 (1889)

Synonyms: *Picoa carthusiana* Tul. & Tul., Fungi hypog, ed. alt.: XXIV (1862), *L. ophthalmosporum* Quél., C. r. Assoc. Franç. Avancem. Sci. 11: 404 (1883) [1882]

3.1.1. Known Localities in Poland (Figure 1A)

Fd-38 KRAKÓW-CZESTOCHOWA UPLAND, Olkusz Upland: Trzyciąż planned reserve, in mixed forest (*Abies*, *Picea*, *Acer pseudoplatanus*), 400 m a.s.l., September 2013 (Chachuła, 2018).

3.1.2. New Localities (Figure 1A)

Bf-47 EASTERN SUDETES, Śnieżnik Mt: Kletno, in mixed forest (*Picea*, *Fagus*), 710 m a.s.l., 2013-07-20, leg. & det. M. Kozak & K. Kozłowska-Kozak, KRA F-2013-35.

Gd-17 OUTER WESTERN CARPATHIANS, **Beskid Żywiecki Mts**, Babia Góra Range, Skawica, in the middle part of the Skawiczanka Stream Valley, in mixed forest (*Abies*, *Fagus*, *Picea*), 550 m a.s.l., 2012-06-28, leg. & det. M. Kozak, KRA F-2012-38.

Gd-59 CENTRAL WESTERN CARPATHIANS, **Western Tatra Mts**: Tatra National Park:

- Wielka Sucha Dolina Valley, in coniferous forest (*Abies*, *Picea*), on calcareous bedrock, 970 m a.s.l., 2019-09-24, leg. & det. M. Kozak & F. Karpowicz, KRA F-TPN/19/0357;
- N slope of Hruby Regiel, above road from Zakopane, in coniferous forest (*Abies*, *Picea*), under *Abies*, 980 m a.s.l., 2019-10-15, leg. & det. M. Kozak & J. Brańka, KRA F-TPN/19/0471.

Ge-11 OUTER WESTERN CARPATHIANS, **Gorce Mts**: mouth of Za Palacem Stream, in garden near buildings, close to the forest edge (*Abies*, *Picea*, *Fagus*), 650 m a.s.l., 2015-11-09, leg. E. Zając, det. P. Mleczek, KRA F-2015-3.

Ge-21 OUTER WESTERN CARPATHIANS, **Gorce Mts**:

- Gorce National Park, upper part of Duże Jaszczce Stream Valley, in Carpathian beech forest (*Dentario glandulosae-Fagetum* association), 790 m a.s.l., 2015-08-17, leg. & det. M. Kozak, KRA F-2015-36;
- Left slope in the middle part of the Forędówki Stream Valley, in mixed forest (*Abies*, *Picea*, *Fagus*), on steep slope by the small stream, 860 m a.s.l., 2012-11-10, leg. & det. M. Kozak & K. Kozłowska-Kozak, KRA F-2012-112.

3.1.3. Notes

The features of the Polish specimens correspond to those published in other studies, for example, Montecchi and Sarasini (2000) and Van Vooren (2017). Tuber-like sporocarps, which can reach over 5 cm in diameter, vinaceous-black-to-black peridium with a granular-to-warty surface, and cream-colored, solid gleba that turns gray with age in fertile chambers, are macroscopic characters of the species (Figure 2A). However, the most distinguishing features are lemon-shaped, deeply yellow-to-yellow-brown, guttulate spores with dimensions of approximately $65\text{--}70 \times 20\text{--}35 \mu\text{m}$ (Figure 2E,F).

Originally described as a new species of *Picoa* by Tulasne & Tulasne (1862), the species was then classified within the genus *Leucangium* by Paoletti (Saccardo, 1889). Recent phylogenetic studies based on multilocus sequence analysis have confirmed its position within the Morchellaceae-Discinaceae lineage in the Morchellaceae family, whereas species of *Picoa*, such as *P. lefebvrei* and *P. juniperina*, have been placed in Pyronemataceae (O'Donnel et al., 1997; Sbissi et al., 2010; Trappe et al., 2010).

The first record of this species in Poland comes from the Olkusz Upland, a calcareous region in the central part of the country (Chachuła, 2018). Several other localities were discovered in southern Poland, mostly in the Carpathians but also in the Sudetes (see the list above). The species is associated in Europe with the coniferous trees *A. alba* and *P. abies* (e.g., Montecchi & Sarasini, 2000). However, in North America, it is a mycorrhizal partner of *Pseudotsuga menziesii* (Palfner & Agerer, 1998; Trappe, 1971; Trappe et al., 2010). In North Africa, *L. carthusianum* grows near *Rhanterium suaveolens* and *Helianthemum lipii* in calcareous alkaline soils, but it could still be a separate taxon (Thomas et al., 2019).

The species is widespread but rare in Europe (Montecchi & Sarasini, 2000; Van Vooren, 2017). Most reports regarding its taxonomy and distribution are provided by MycoDB [“Base de Jacques Trimbach (*Picoa carthusiana*)” 2020], Oertel (2011), and Van Vooren (2017). Other than Southern and Western European countries, such as France (Van Vooren, 2017), Italy (Montecchi & Sarasini, 2000), Spain, and Switzerland (Flammer, 2011; Senn-Irlet, 1985), it has also been found in all Central European countries, including Austria (“5976. *Picoa carthusiana* Tul.,” 2020), the

Czech Republic (Valda, 2018; Veselý, 1928), Germany (“*Picoa carthusiana* Tul. & C. Tul. 1862,” 2020), Hungary (Bratek et al., 2013), Slovakia (Glejdura, 2013; nahuby.sk, 2020; Pejger, 2019), northern Romania (Béres, 1999), and Poland. Most localities are present in mountain ranges, for example, the Alps in southern Germany and Austria, the Sudetes in southeastern Germany and Poland, and the Carpathians in Hungary, Romania, Slovakia, and southern Poland. However, it has also been found in low elevation areas, such as the Moravia and Prague regions in the Czech Republic and the Olkusz upland in Poland. It has been recorded in both calcareous regions, such as the Olkusz upland, Tatras, and Alps, and on acid, volcanic bedrocks, for example, the Stolické vrchy in Slovakia (Glejdura, 2013) and the Sudetes.

3.2. *Rhizopogon melanogastroides* M. Lange, Beihefte zur Sydowia 1: 255 (1957)

Synonyms: -

3.2.1. New Localities (Figure 1B)

Gd-59 CENTRAL WESTERN CARPATHIANS, **Western Tatra Mts:** Tatra National Park: left slope of Strážyska Valley, on a limestone rock, under *Pinus mugo*, 1,130 m a.s.l., 2019-10-14, leg. M. Kozak & J. Braňka, det. P. Mleczo, KRA F-TPN/19/0056.

3.2.2. Notes

The species was described by Lange in 1957, who based its description on the specimen collected by Franz Petrak in Moravia. Because of the peculiar bullet-shaped thick-walled pigmented spores, it was believed to occupy a transitional position between the genera *Rhizopogon* and *Melanogaster* (Lange, 1957; Martín, 1996; Trappe, 1975). In the monograph of the European species of *Rhizopogon*, Martín (1996) included *R. melanogastroides* in this genus; however, phylogenetic analysis based on the ITS nrDNA region suggested that the taxon should be placed outside *Rhizopogon* (Martín & Raidl, 2002). The results of molecular studies as well as the features of the ectomycorrhizas were arguments against including this species in the genus *Melanogaster*. Indeed, although peculiar due to the presence of globular cells in the outer mantle, the ectomycorrhizas of *R. melanogastroides* share some important features with other *Rhizopogon* species, that is, a lack of sclerotia, clamps, and globular to capitate cystidia (typical for *Melanogaster ectomycorrhizas*) and the presence of a gelatinous matrix in the outer mantle (Agerer, 2006). In the phylogenetic analyses based on the LSU nrDNA region, performed by Binder and Hibbett (2006), it was found to be nested within the genus *Rhizopogon*.

This species is extremely rare in Europe. Long known only from the historical type locality in Moravia, in the municipality of Hranice (German: Mährisch Weißkirchen), and in the Moravian Gate between the Carpathians and the Sudetes (Lange, 1957; Martín, 2001), it was then discovered in 1997 in the Bad Tölz-Wolfratshausen District, Bavaria, Germany, in the Northern Limestone Alps (Martín & Raidl, 2002; Raidl et al., 1998) and 10 years later, in 2007, in Cancano, the province of Sondrino, Lombardy, Italy, in the Southern Limestone Alps (Bincoletto, 2014). In all three localities, sporocarps were found in limestone areas under *Pinus*: in the Czech Republic – in open slopes, under undetermined *Pinus* species, in a mixed wood dominated by *Quercus* sp. (Lange, 1957); in Germany, in a loose stand of *Picea abies*, *Pinus sylvestris*, *P. mugo*, and some shrubs (*Erico-Pinetum* plant association), close to the bank of the river Isar, at 795 m a.s.l. (Martín & Raidl, 2002); in Italy, in an alpine grassland with *Dryas octopetala*, *Salix serpyllifolia*, *Gentianella ciliata*, *G. campestris*, *Leontopodium alpinum*, and *Chamorchis alpina*, at ca. 2,000 m a.s.l. (Bincoletto, 2014). The Polish locality is also placed in an exposed limestone rock with scattered *P. mugho* at 1,130 m a.s.l. in the mountain valley.

Lange based his description of the new species on exsiccatum, which consisted of one well-preserved sporocarp, deposited in the Botanical Museum of the University of Copenhagen (Museum Botanicum Hauniense). Macromorphological characters were then completed by the description of fresh sporocarps from Germany and Italy (Bincoletto, 2014; Martín & Raidl, 2002; see Table 1). The study of Italian

sporocarps revealed a strong dextrinoid reaction of young, hyaline spores that cannot be observed in mature, pigmented ones. Analysis of sporocarps from the Tatra Mts confirms this statement (Table 1, Figure 3D). We also found no bluing reaction of mature sporocarps after touching, reported for the material collected in Germany (Martín & Raidl, 2002). However, we found a reddish peridium reaction after the application of 10% KOH, as reported by Bincoletto (2014), which can also be tested in dry specimens. Macro- and microcharacters of specimens from Tatra Mts are generally in agreement with those presented in previous studies (Table 1, Figure 2C,D, Figure 3C–H).

3.3. *Melanogaster luteus* Zeller, Mycologia 31(1): 9 (1939)

Synonyms: *Alpova luteus* Trappe, Beihefte zur Nova Hedwigia 51: 291 (1975), *Melanogaster microsporus* Mattir., Beiträge zur Kryptogamenflora der Schweiz 8(2): 39 (1935), *Alpova diplophloeus* f. *europaeus* Trappe, Beihefte zur Nova Hedwigia 51: 289 (1975)

3.3.1. New Localities (Figure 1C)

Ge-50 CENTRAL WESTERN CARPATHIANS, **Western Tatra Mts:** Tatra National Park: S slope of Kopieniec Wielki Mt, artificial stand (*Alnus incana*, *Larix* sp.) in a calcareous sward representing *Seslerion tatrae* alliance, 1,300 m a.s.l., 2019-08-26, leg. F. Karpowicz & K. Kozłowska-Kozak, det. P. Mleczo, Filip Karpowicz, KRA F-TPN/19/0006.

Ge-51 CENTRAL WESTERN CARPATHIANS, **High Tatra Mts:** Tatra National Park: mouth of Waksmundzki Stream, *Alnetum incanae* association (*Alnus incana*, *Picea*), 1,000 m a.s.l., 2019-09-21 (found in two localities), leg. & det. M. Kozak, KRA F-TPN/19/0291, KRA F-TPN/19/0292.

Gf-46 OUTER EASTERN CARPATHIANS, **Western Bieszczady Mts:** Łubne, mouth of the Kotanica Stream, in *Alnetum incanae* association (*Alnus incana*, *Tilia*, *Corylus*), 520 m a.s.l., 2012-10-22, leg. M. Kozak & K. Kozłowska-Kozak, det. M. Kozak & P. Mleczo, KRA F-2012-86.

3.3.2. Notes

The taxonomy of the *Alnus*-associated species of Boletales related to *Paxillus*, that is, *Alpova* and *Melanogaster*, has long been very complicated and unclear due to uncertain descriptions and misleading interpretations of names and species concepts (Moreau et al., 2011). For that reason, reconstruction of the distribution of species in this group is difficult and often requires the study of original collections. This is also true for *M. luteus*, which is often referred to as *Alpova diplophloeus*, especially if specimens are identified according to Kers (1986), Knudsen (2012), Montecchi and Lazzari (1989), and Montecchi and Sarasini (2000). Another name used for this taxon in the European keys is *M. microsporus* (e.g., Eckblad & Lange, 1997).

The features of the Carpathian specimens correspond well with the description published by Moreau et al. (2011). Small, round-to-slightly flattened sporocarps of up to 1.5 cm in diameter without columella, greenish-yellow-to-greenish-brown, a furfuraceous-to-stringy peridium surface, black, deliquescent, mature gleba with whitish-to-pale yellow septa are characteristic macromorphological features of this species (Figure 2B). In the micromorphological aspect, the most distinguishing features are: peridium that is plectenchymatous throughout (pseudoparenchymatous in the inner layer in *Alpova*) (Figure 3A,B), yellow-brown, elongated spores $5.3\text{--}6.8 \times 2.4\text{--}3.3 \mu\text{m}$ with remnants of hyaline sterigmata at their bases (Figure 2G,H), basidia with thin walls and lack of spherical “supporting cells” in the gleba (characteristic for *Alpova* spp.). The closely related species *M. rivularis*, known to date only from Corsica, differs in bigger sporocarps, over 1.5 cm in diameter, with distinct columella and basidia with thick walls at their apices (Moreau et al., 2011).

The presence of *M. luteus* was confirmed in France, Italy, Montenegro, and Scandinavia; however, it is most likely to be more frequent but rarely searched for (Moreau et al., 2011). The only confirmed localities in Central Europe are those in Slovakia (nahuby.sk, 2020), and now its presence has been confirmed in Poland in the Central Western and Outer Eastern Carpathians. The Polish locality in

the Bieszczady Mts is the most extreme eastern location known so far. The most frequent mycorrhizal partner of *M. luteus* is *Alnus incana* in alluvial places in rivers or stream banks in mountainous areas (Moreau et al., 2011).

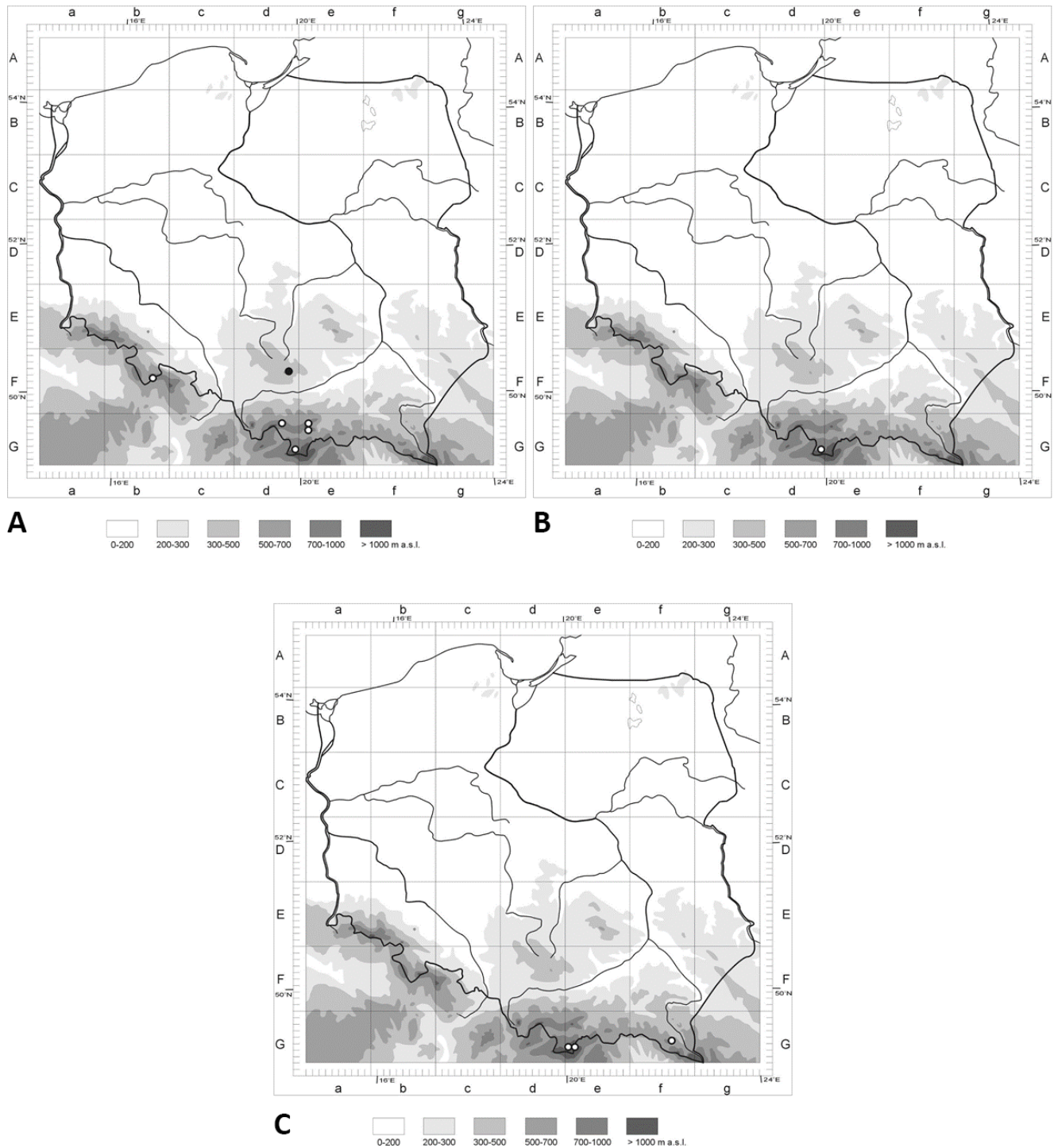


Figure 1 Distribution maps of three hypogeous fungi in Poland: (A) *Leucangium carthusianum*; (B) *Rhizopogon melanogastroides*; (C) *Melanogaster luteus*. The points correspond to the ATMOS squares with localities (see the lists of localities). White dots – new localities; black dot – known locality.

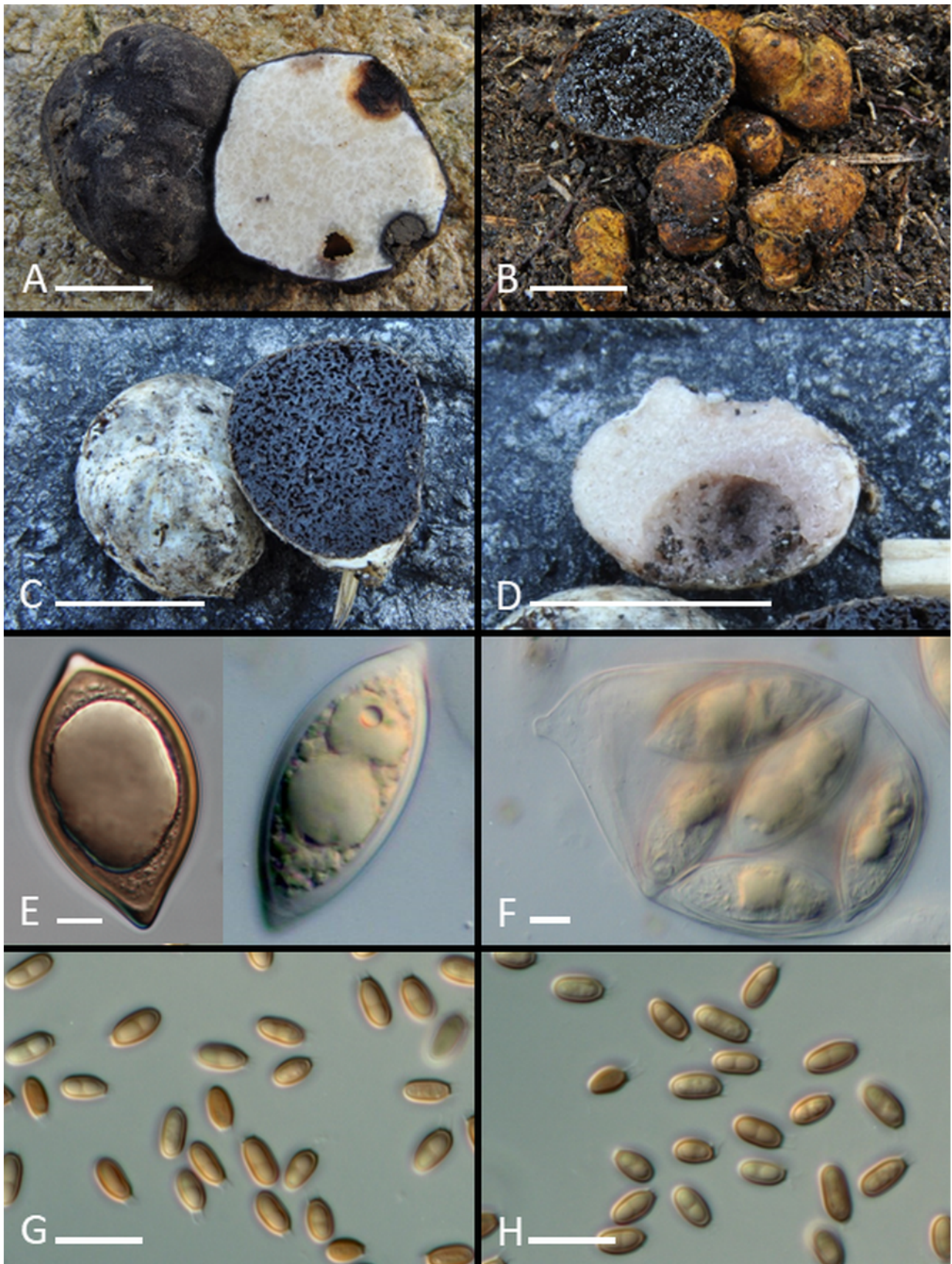


Figure 2 Sporocarps and spores of hypogeous fungi: (A) Sporocarp of *Leucangium carthusianum*; (B) sporocarps of *Melanogaster luteus*; (C,D) mature (C) and immature (D) sporocarps of *Rhizopogon melanogastroides*; (E) spores of *L. carthusianum*; left – spore from fresh specimen, right – spore from dry specimen (8-years old); (F) ascus of *L. carthusianum*; (G,H) spores of *M. luteus*. Scale: (A–D) 10 mm; (E–H) 10 µm. Photo: (A–D) M. K.; (E–H) P. M. (A) KRA F-TPN/19-0357; (B,G,H) KRA F-TPN/19-0006; (C,D) KRA F-TPN/19-0056; (E,F) KRA F-2012-38.

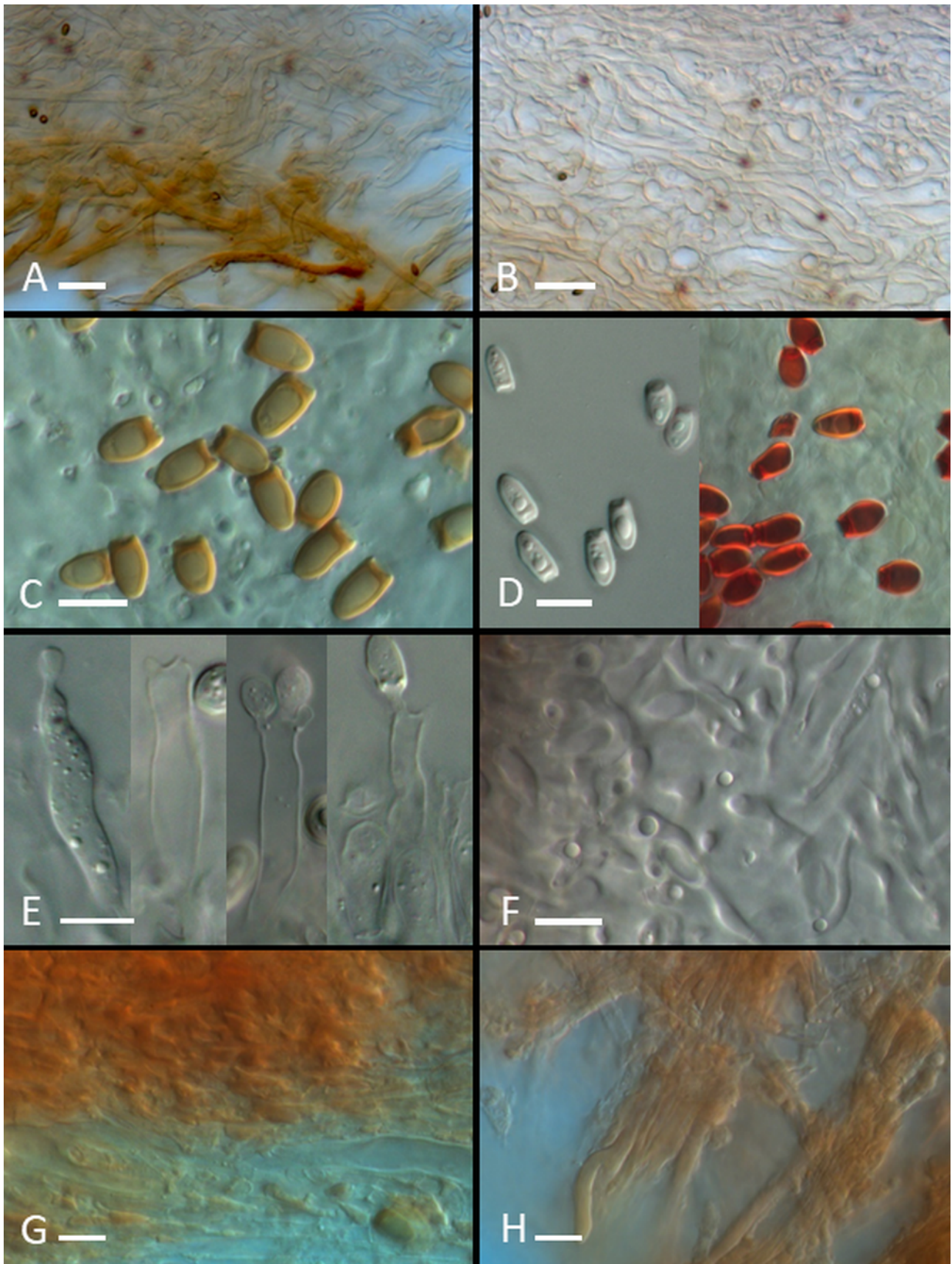


Figure 3 Micromorphological structures of hypogeous fungi: (A,B) Cross section of outer (A) and middle (B) peridium layers of *Melanogaster luteus*; (C,D) mature (C) and immature (D) spores of *Rhizopogon melanogastroides* (left – in water, right – in Melzer's reagent); (E) basidia of *R. melanogastroides* (first from the left – immature basidium); (F) section of dissepiment of *R. melanogastroides*; (G,H) section of the inner (G) and outer (H) peridium layers of *R. melanogastroides*. Scale: 10 μ m. Photo: P. M. (A,B) KRA F-TPN/19-0006; (C-H) KRA F-TPN/19-0056.

Table 1 A synoptic table of macro- and microcharacters and chemical reactions of *Rhizopogon melanogastroides* according to descriptions of specimens from the Czech Republic (Lange, 1957), Germany (Martín, 1996; Martín & Raidl, 2002), Italy (Bincoletto, 2014), and Poland (the present study).

	Czech Republic (based on exsiccatum)	Germany	Italy	Poland*
Macromorphology				
Sporocarp – shape	Almost regular	Young subglobose to oval, mature oval to elongated, to some degree irregular	Irregular in shape, more or less spherical, but also reniform, knotted-lobed	Young subglobose, mature oval, elongated
Sporocarp – dimensions	18 × 12 mm	Fresh specimens: 17 × 10 × 8 mm (the biggest)	Fresh specimens: 10–25 mm (diameter)	Fresh specimens: young 9 × 12 mm, mature 16 × 13 mm
Rhizomorphs	Not mentioned (or as innate fibrils close to base)	Some at the base	With rare residues at the base	One at the base
Peridium – surface and color (fresh specimens)	Not observed	Smooth; young: creamy whitish to dull yellowish, older: dark dull yellow to pale brown, with gleba shining through in some places	Cottony, felted; white, in some areas translucent, with gleba shining through	Cottony, felted; whitish, in some areas translucent, with gleba shining through in some places
Peridium – color changes at exposure or manipulation (fresh specimens)	Not observed	Young: brown a while after touching; mature: bluing (pale to gray-bluish) immediately after touching, after a while changes to dark reddish, then woody brown	Pinkish-brown	Pinkish, then brownish
Peridium – color of exsiccata	Pinkish buff to pale cinnamon buff or more yellowish, darker (cinnamon brown) around the base; almost black innate fibrils present near the base	Dull dark brown (without any red)	Not specified	Ochre, pale cinnamon to brownish
Gleba	Firm, chambers irregular, small pale wood brown, septa thick, milky-white	Firm, chambers irregular, young: creamy whitish to yellowish, mature: deep black; exsiccata: dark brown to woody brown	Chambers irregular, round to labyrinthine, 1 to 3 mm in diameter, young: pinkish, mature: blackish-brown; septa grayish; exsiccata: not specified	Firm, chambers irregular, labyrinthine, young: creamy whitish to pinkish, mature: blackish-brown; septa grayish, glassy; in exsiccata: very dark brown, septa dark gray, glassy
Smell	Not tested	Young: unspecific, mature: distinct, sweetish, resembles fruit (cherries in alcohol)	Clear and persistent of ripe fruit	Not checked
Macrochemical reactions	Not checked	Not checked	KOH 5% – peridium vermilion red, FeSO ₄ – peridium slowly greenish; gleba – no reaction	KOH 10% – peridium pinkish-red (fresh and dry specimen)

Continued on next page

Table 1 continued

	Czech Republic (based on <i>exsiccatum</i>)	Germany	Italy	Poland*
Micromorphology				
Peridium structure	60–90 µm thick (revived), inner layer of thin densely arranged hyphae, with large pigment balls, tawny or ochraceous tawny, outer part of coarser and more loosely woven hyphae	Up to 225 µm thick, inner layer formed by compact and parallel running hyphae (roseolus-type), outer layer formed by interwoven hyphae (abietis-type), hyphae 5.5–6 µm in diameter, hyaline to yellowish, with numerous orange-brown pigment deposits	Variable in thickness, from 50 to 100 µm, up to 200–250 µm, composed of irregular intertwined, encrusted, trichoderm-like hyphae, simple-septated, 5–6 µm broad, with yellowish pigment similar to that found in <i>R. roseolus</i> and <i>R. abietis</i>	Variable in thickness, up to ca. 300 µm, inner layer formed by compact and parallel running hyphae (roseolus-type), outer layer formed by interwoven hyphae, some running parallel (abietis-type), hyphae of inner layer 1.8–3.9–8.7 µm in diameter, outer layer 1.6–2.9–4.3 µm in diameter, hyaline to yellowish, with numerous orange-brown pigment deposits staining reddish in 3% KOH
Septa (trama plates, dissepiments) structure	50–150(350) µm thick, not sessile, build-up of interwoven hyphae arranged subregularly in central part of some septa, 3 µm broad, slightly or not gelatinous, several of them with many small oil drops, somewhat refractive, much branched, sparingly septate, hyaline, a few pigment balls occasionally seen in parts near peridium	Septa up to 100 µm thick, composed of interwoven, thin-walled, refractive, branched and septated hyphae, 3.5–4 µm in diameter	Not specified	Septa up to 150 µm thick, composed of interwoven, hyaline, thin-walled, branched and septated hyphae immersed in a hyaline matrix, hyphae 1.2–3.7–5.9 µm in diameter
Hymenium	Basidia (few seen) with up to eight spores, sterigmata short and thick; paraphyses not distinctly gelatinized, hyaline, 25 × 6–7 µm	Brachybasidioles 28–32 × 4.5–5 µm, hyaline, thin-walled	Not specified	Basidia cylindrical to clavate, with up to eight spores, 24–35 × 5–6 µm, sterigmata short, brachybasidioles clavate, thin-walled
Spores	Projectile-shaped, broadest near base, apex blunt, base truncate with a large basal chamber, very thick-walled, with one or few small oil drops, individual spores hyaline, spore mass pale olive buff, variable in size, 7.7–10.9 × 4.7–6.1 µm	Elliptical to oblong, broadly truncate with inverted cup-shaped base, 7.5–10 × (3.8)–4.5–6.6 µm, thick-walled (1 µm), yellowish to pale ochre	Irregularly shaped, tapering towards the tip, elongated, base truncate dextrinoid (best seen in juvenile spores), with a basal chamber, spores hyaline, average dimensions 7–10(12) × 3.5–4.5(6) µm	Projectile-shaped, broadest near base, apex blunt, base truncate with basal chamber, thick-walled (0.6–1.1 µm), base thicker (up to 2 µm), most with one oil drop, individual spores hyaline when immature, then yellowish to yellow, base orange-yellow, 7.3–9.5–11.8 × 4.8–5.6–6.4 µm; young spores distinctly dextrinoid

* Values of microscopic measurements are given as: minimum–mean–maximum.

4. Discussion and Conclusions

Investigations carried out in recent years have identified new localities of some hypogeous fungi, which are new for the mycobiota or have not been recorded for over 50 years in Poland. Most species, e.g., *E. aculeatus*, *Gautieria trabutii*, *Hydnotrya bailii*, *Hymenogaster luteus*, *Hysterangium nephriticum*, *Lactarius stephensii*, *Melanogaster tuberiformis*, *Octaviania lutea*, *Pachyphlodes conglomerata*, *P. citrina*, *Russula candidissima*, *R. mattirolana*, *Sclerogaster hysterangioides*, *Tuber bellonae*, *T. excavatum*, *T. ferrugineum*, *T. fulgens*, and *T. macrosporum*, have been found either in the Carpathians or in the upland regions in the middle and middle-eastern part of Poland (the Śląsko-Krakowska Upland, Małopolska Upland, Lubelsko-Lwowska Upland, and Polesie region) (Chachuła et al., 2020; Gierczyk et al., 2019; Hilszczańska et al., 2008, 2013, 2014; Kujawa & Gierczyk, 2011; Ławrynowicz, 2009; Ławrynowicz et al., 2008; Rutkowski et al., 2014; Vidal et al., 2019; Wojewoda et al., 2016). Reports on single species have also come from other regions, such as the Eastern Sudetes (*R. cerea*), Lubuskie Lake District (*Gautieria otthii*), or Gdańsk and Wschodniopomorskie Lake District (*Melanogaster macrosporus*) (Kujawa & Gierczyk, 2011, 2013; Ślusarczyk, 2018; Vidal et al., 2019). Such geographical patterns reflect the activity of researchers; however, data regarding the distribution of hypogeous fungi in Poland seem to indicate that the regions mentioned above, that is, the Carpathian Mts and the calcareous uplands in the central and southeastern parts of the country, are hot spots of diversity for this fungal group in Poland.

In this study, we present new localities of three species, two of which are *M. luteus* and *R. melanogastroides*, which are new for Poland. Both were recorded in the Carpathian Mts and both appear to have specific and rather narrow ecological requirements. In the most recent records in Europe, including the Polish record, *R. melanogastroides* was found only in the mountain habitats on calcareous bedrock, in association with the dwarf pine *P. mugho*. The occurrence of *M. luteus* in Europe is restricted by its association with its mycorrhizal partner, the genus *Alnus*, specifically *A. incana*, a species characteristic of stream bank habitats in mountain areas. Indeed, Polish localities of *M. luteus* fit well with this distribution pattern. In contrast to the above-mentioned species, the sporocarps of *L. carthusianum* were found in mixed and coniferous forests not only in mountain regions but also in the Kraków-Częstochowa Upland. The species seems to be associated primarily with *Abies alba*, a tree found in almost all known localities of *L. carthusianum*; however, *Picea* is the second possible mycorrhizal partner of this species, as it is the only coniferous tree found in the locality in the Sudetes.

The data presented in this article broaden our knowledge regarding the diversity and distribution of hypogeous fungi in Poland. Together with recent findings, our records indicate that the entire hypogeous fungal diversity of the Polish mountains and uplands has not yet been discovered, and new findings can be expected in future.

Acknowledgments

The authors wish to express their gratitude to the Tatra National Park authorities for their support of this study on hypogeous fungi in the area of the Tatra Mts.

References

5976. *Picoa carthusiana* Tul. (2020). Datenbank der Pilze Österreichs. Retrieved July 8, 2020, from http://austria.mykodata.net/Taxa_sites.aspx?qvntaxidTaxon=277438
- Agerer, R. (2006). Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress*, 5, 67–107. <https://doi.org/10.1007/s11557-006-0505-x>
- Alvarado, P., Cabero, J., Moreno, G., Bratek, Z., Vooren, N. V., Kaounas, V., Konstantinidis, G., Agnello, C., Merényi, Z., Smith, M., Vizzini, A., & Trappe, J. M. (2016). Phylogenetic overview of the genus *Genea* (Pezizales, Ascomycota) with an emphasis on European taxa. *Mycologia*, 108(2), 441–456. <https://doi.org/10.3852/15-199>
- Base de Jacques Trimbach (Picoa carthusiana)*. (2020). MycoDB. Retrieved July 8, 2020, from <https://www.mycodb.fr/trimbachfull.php?query=Picoa%20carthusiana&action=search>

- Béres, M. (1999). Data to the vegetation of mushrooms in the Upper-Tisa Region. *Tisia Monograph Series*, 4, 293–307.
- Bincoletto, A. (2014). Un raro *Rhizopogon* del piano alpino: *R. melanogastroides* [A rare *Rhizopogon* from the Alpine zone: *R. melanogastroides*]. In M. D. Maggiora, L. Gori, & U. Pera (Eds.), *Atti di Convegno Micologico Nazionale di ricerca e studio dei funghi ipogei della Lucchesia* [Proceedings of the national mycological conference on research and study of hypogeous fungi of Lucchesia] (pp. 43–47). Gruppo Micologico “M. Danesi”.
- Binder, M., & Hibbett, D. S. (2006). Molecular systematics and biological diversification of Boletales. *Mycologia*, 98(6), 971–981. <https://doi.org/10.1080/15572536.2006.11832626>
- Bratek, Z., Merényi, Z., & Varga, T. (2013). Changes of hypogeous fungi in the Carpathian-Pannonian region in the past centuries. *Acta Mycologica*, 48(1), 33–39. <https://doi.org/10.5586/am.2013.005>
- Chachuła, P. (2018). Grzyby wielkoowocnikowe Dłubniańskiego Parku Krajobrazowego – wstępne wyniki badań [Macrofungi in the Dłubnia Landscape Park – Preliminary results]. *Przegląd Przyrodniczy*, 29(2), 58–71.
- Chachuła, P., Fiedor, M., Rutkowski, R., & Dorda, A. (2020). New records to the mycobiota of the Cieszyn municipality (Polish Western Carpathians) including new species for Poland. *Acta Mycologica*, 55(1), Article 5511. <https://doi.org/10.5586/am.5511>
- Chmiel, A. (2006). *Checklist of Polish Larger Ascomycetes*. W. Szafer Institute of Botany, Polish Academy of Sciences.
- Eckblad, F. E., & Lange, M. (1997). Melanogastrales Svrček. In L. Hansen & H. Knudsen (Eds.), *Nordic Macromycetes* (Vol. 3, pp. 294–295). Nordsvamp.
- Flammer, R. (2011). St. Galler Hypogäen [Hypogeous fungi of St. Gallen]. *Schweizerische Zeitschrift für Pilzkunde*, 89(2), 66–67.
- Gierczyk, B., Kujawa, A., Szczepkowski, A., Ślusarczyk, T., Pachlewski, T., Chachuła, P., & Domian, G. (2019). Macrofungi of the Bieszczady Mountains. *Acta Mycologica*, 54(2), Article 1124. <http://doi.org/10.5586/am.1124>
- Glejdura, S. (2013). Nové nálezy bazidiových a vreckatých húb v Stolických vrchoch (Slovensko) [New records of basidiomycetes and ascomycetes in the Stolické vrchy (Slovakia)]. *Mykologické Listy*, 124, 15–40.
- Hilszczańska, D., Rosa-Gruszecka, A., Sikora, K., & Szmidla, H. (2013). First report of *Tuber macrosporum* occurrence in Poland. *Scientific Research and Essays*, 8(23), 1096–1099.
- Hilszczańska, D., Rosa-Gruszecka, A., & Szmidla, H. (2014). Characteristic of *Tuber* spp. localities in natural stands with emphasis on plant species composition. *Acta Mycologica*, 49(2), 267–277. <http://doi.org/10.5586/am.2014.024>
- Hilszczańska, D., Sierota, Z., & Palenzona, M. (2008). New *Tuber* species found in Poland. *Mycorrhiza*, 18, 223–226. <https://doi.org/10.1007/s00572-008-0175-4>
- Kers, L. E. (1986). Några Norska fynd av hypogéer [Some Norwegian findings of hypogeous fungi]. *Agarica*, 7(14), 30–48.
- Knudsen, H. (2012). Alpova C. W. Dodge. In H. Knudsen & J. Vesterholt (Eds.), *Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gasteroid genera* (p. 212). Nordsvamp.
- Kozak, M., Mleczo, P., & Ławrynowicz, M. (2013). The problem of rarity of hypogeous fungi on the example of *Octaviania asterosperma* and *Chamonixia caespitosa* (Boletales). In A. Biedunkiewicz & M. Dynowska (Eds.), 56. Zjazd Polskiego Towarzystwa Botanicznego, Olsztyn, 24–30 czerwca 2013. Streszczenia wystąpień ustnych i plakatów [56 Conference of the Polish Botanical Society, Olsztyn, 24–30 June 2013. Abstracts of oral and poster presentations] (pp. 327–328). Polish Botanical Society.
- Kujawa, A., & Gierczyk, B. (2011). Rejestr gatunków grzybów chronionych i zagrożonych w Polsce. Część IV. Wykaz gatunków przyjętych do rejestru w 2008 roku [Register of protected and endangered fungi species in Poland. Part IV. A list of species recorded in 2009]. *Przegląd Przyrodniczy*, 22(1), 17–83.
- Kujawa, A., & Gierczyk, B. (2013). Rejestr gatunków grzybów chronionych i zagrożonych w Polsce. Część VII. Wykaz gatunków przyjętych do rejestru w 2011 roku [Register of protected and endangered fungi species in Poland. Part VII. A list of species recorded in 2011]. *Przegląd Przyrodniczy*, 24(2), 3–44.
- Lange, M. (1957). *Rhizopogon melanogastroides* n. sp. *Sydowia*, 1, 255–256.
- Ławrynowicz, M. (1988). *Ascomycetes, Elaphomycetales, Tuberales. Flora Polska, Grzyby* [Flora of Poland, Fungi] (Vol. 18). Państwowe Wydawnictwo Naukowe.
- Ławrynowicz, M. (2009). Four *Tuber* species accompanying *T. mesentericum* in natural sites in Poland. *Anales del Jardín Botánico de Madrid*, 66S1, 145–149. <https://doi.org/10.3989/ajbm.2227>
- Ławrynowicz, M., Krzyszczyk, T., & Fałdziński, M. (2008). Occurrence of black truffles in Poland. *Acta Mycologica*, 43(2), 143–151. <https://doi.org/10.5586/am.2008.018>

- Martín, M. P. (1996). *The genus Rhizopogon in Europe*. Catalanian Mycological Society.
- Martín, M. P. (2001). Chorologic database of European *Rhizopogon* species. *Mycotaxon*, 78, 191–244.
- Martín, M. P., & Raidl, S. (2002). The taxonomic position of *Rhizopogon melanogastroides* (Boletales). *Mycotaxon*, 84, 221–228.
- Mleczek, P., Kozak, M., Ławrynowicz, M., & Dubiel, G. (2010). *Octaviania asterosperma* (hypogeous Basidiomycota). Recent data to ecology and distribution. *Acta Mycologica*, 45(2), 133–144. <https://doi.org/10.5586/am.2010.017>
- Mleczek, P., Kozak, M., Ławrynowicz, M., & Górszczyk, A. (2009). New localities of *Chamonixia caespitosa* (hypogeous Boletaceae) in Central Europe. *Acta Mycologica*, 44(1), 29–42. <https://doi.org/10.5586/am.2009.005>
- Montecchi, A., & Lazzari, G. (1989). Melanogastraceae species collected in the Appennino Reggiano-Parmense area. *Micologia Italiana*, 18(2), 33–48.
- Montecchi, A., & Sarasini, M. (2000). *Funghi ipogei d'Europa* [European hypogeous fungi]. Associazione Micologica Bresadola; Fondazione Centro Studi Micologici.
- Moreau, P. A., Rochet, J., Richard, F., Chassagne, F., Manzi, S., & Gardes, M. (2011). Taxonomy of *Alnus*-associated hypogeous species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe. *Cryptogamie, Mycologie*, 32(1), 33–62. <https://doi.org/10.7872/crym.v32.iss1.2012.033>
- nahuby.sk*. (2020). Retrieved July 8, 2020, from <https://www.nahuby.sk/>
- O'Donnell, K., Cigelnik, E., Weber, N. S., & Trappe, J. M. (1997). Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *Mycologia*, 89(1), 48–65. <https://doi.org/10.1080/00275514.1997.12026754>
- Oertel, B. (2011). *Notizbuchartige Auswahlliste zur Bestimmungsliteratur für Discomyceten und hypogäische Ascomyceten* [Notebook-like selection list for identification of Discomycetes and hypogeous Ascomycetes]. Deutsche Gesellschaft für Mykologie. <https://www.dgfm-ev.de/publikationen/weitere-publikationen/oertel-2008-pilzgattungen-europas?name=Lit-Discomyceten.pdf&reattachment=5c81ad0b03c58b79800ccc5557cd4e44>
- Palfner, G., & Agerer, R. (1998). *Leucangium carthusianum* (Tul.) Paol. + *Pseudotsuga menziesii* (Mirb.) Franco. *Descriptions of Ectomycorrhizae*, 3, 37–42.
- Paz, A., Bellanger, J. M., Lavoise, C., Molia, A., Ławrynowicz, M., Larsson, E., Ibarra, I. O., Jeppson, M., Læssøe, T., Sauve, M., Richard, F., & Moreau, P. A. (2017). The genus *Elaphomyces* (Ascomycota, Eurotiales): A ribosomal DNA-based phylogeny and revised systematics of European “deer truffles”. *Persoonia*, 38, 197–239. <https://doi.org/10.3767/003158517X697309>
- Paz, A., Vidal, J. M., Lavoise, C., & Moreau, P. A. (2016). Revisión taxonómica del género *Octaviania* (Boletales) en Europa [Taxonomic revision of the genus *Octaviania* (Boletales) in Europe]. *Boletín Micológico de la FAMCAL*, 11, 101–138.
- Pejger, M. (2019). Mykoflóra Tatranského národného parku a jeho ochranné pásma [Mycoflora of the Tatra National Park and its protection zones]. *Spravodajca Slovenskej mykologickej spoločnosti*, 50, 6–18.
- Picoa carthusiana* Tul. & C. Tul. 1862. (2020). Deutsche Gesellschaft für Mykologie. Retrieved July 8, 2020, from <http://www.pilze-deutschland.de/organismen/picoa-carthusiana-tul-c-tul-1862-1>
- Raidl, S., Beenken, L., & Agerer, R. (1998). *Rhizopogon melanogastroides* M. Lange + *Pinus mugo* Turra. *Descriptions of Ectomycorrhizae*, 3, 99–104.
- Rutkowski, R., Chachuła, P., Fiedor, M., & Beczała, T. (2014). Grzyby i śluzowce [Fungi and slime molds]. In R. W. Mysłajek (Ed.), *Monografia przyrodnicza Góry Bucze* [Monograph of Góry Bucze] (pp. 29–36). Gmina Brenna.
- Saccardo, P. A. (1889). Discomyceteae et Phymatosphaeriaceae. *Sylloge fungorum*, 8, 1–1143.
- Sbissi, I., Neffati, M., Boudabous, A., Murat, C., & Gtari, M. (2010). Phylogenetic affiliation of the desert truffles *Picoa juniperi* and *Picoa lefebvrei*. *Antonie van Leeuwenhoek*, 98(4), 429–436. <https://doi.org/10.1007/s10482-010-9456-y>
- Senn-Irlet, B. (1985). *Picoa carthusiana* Tul. – ein seltener hypogäischer Ascomycet [*Picoa carthusiana* Tul. – A rare hypogeous ascomycete]. *Schweizerische Zeitschrift für Pilzkunde*, 63, 216–218.
- Stielow, B., Bratek, Z., Orczán, A. K. I., Rudnoy, S., Hensel, G., Hoffmann, P., Klenk, H. P., & Göker, M. (2011). Species delimitation in taxonomically difficult fungi: The case of *Hymenogaster*. *PLoS ONE*, 6(1), Article e15614. <https://doi.org/10.1371/journal.pone.0015614>
- Ślusarczyk, T. (2018). The first locality of *Gautieria otthii* Trog in Poland. *Przegląd Przyrodniczy*, 29(2), 122–126.

- Thomas, P. W., Elkhateeb, W. A., & Daba, G. (2019). Truffle and truffle-like fungi from continental Africa. *Acta Mycologica*, 54(2), Article 1132. <https://doi.org/10.5586/am.1132>
- Trappe, J. M. (1971). A synopsis of the Carbomycetaceae and Terfeziaceae (Tuberales). *Transactions of the British Mycological Society*, 57(1), 85–92. [https://doi.org/10.1016/s0007-1536\(71\)80083-9](https://doi.org/10.1016/s0007-1536(71)80083-9)
- Trappe, J. M. (1975). A revision of the genus *Alpova* with notes on *Rhizopogon* and the Melanogastraceae. *Beihefte zur Nova Hedwigia*, 51, 270–309.
- Trappe, M. J., Trappe, J. M., & Bonito, G. M. (2010). *Kalapuya brunnea* gen. & sp. nov. and its relationship to the other sequestrate genera in Morchellaceae. *Mycologia*, 102(5), 1058–1065. <https://doi.org/10.3852/09-232>
- Tulasne, L. R., & Tulasne, C. (1862). *Fungi hypogaei. Histoire et monographie des champignons hypoges* [Fungi hypogaei. History and monography of hypogeous fungi] (2nd ed.). F. Kliercksieck.
- Valda, S. (2018). Podzemky přímo pod nosem. O dvou nálezech hlízatky černé (*Picoa carthusiana* Tul. et C. Tul.) v ČR [Hypogeous fungi directly under the nose. About two findings of black tuber (*Picoa carthusiana* Tul. et C. Tul.) in Czech Republic]. *Mykologický sborník*, 2, 35–42.
- Van Vooren, N. (2017). *Leucangium carthusianum* (Pezizales), la truffe de Chartreuse découverte en Auvergne [*Leucangium carthusianum* (Pezizales), the Chartreuse truffle discovered in Auvergne]. *Bulletin Mycologique et Botanique Dauphiné-Savoie*, 226, 33–38.
- Veselý, R. (1928). *Picoa Carthusiana* Tul., hlízatka černá [*Picoa Carthusiana* Tul., black tuber]. *Časopis českých houbařů*, 8, 33–35.
- Vidal, J. M., Alvarado, P., Loizides, M., Konstantinidis, G., Chachuła, P., Mleczko, P., Moreno, G., Vizzini, A., Krakhamnyi, M., Paz, A., Cabero, J., Kaounas, V., Slavova, M., Moreno-Arroyo, B., & Llistosella, J. (2019). A phylogenetic and taxonomic revision of sequestrate russulaceae in Mediterranean and temperate Europe. *Persoonia*, 42, 127–185. <https://doi.org/10.3767/persoonia.2019.42.06>
- Wojewoda, W. (2000). *Atlas of the geographical distribution of fungi in Poland*. W. Szafer Institute of Botany, Polish Academy of Sciences.
- Wojewoda, W. (2003). *Checklist of Polish larger Basidiomycetes*. W. Szafer Institute of Botany, Polish Academy of Sciences.
- Wojewoda, W., Kozak, M., Mleczko, P., & Karasiński, D. (2016). *Grzyby makroskopijne Gorców (Karpaty Zachodnie)* [Macrofungi of the Gorce Mts (Western Carpathians)]. Instytut Botaniki im. W. Szafera, Polska Akademia Nauk.