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First records of *Melanoleuca galbuserae* (*Melanoleucaceae*, *Basidiomycota*) from Spain and notes on its ecology

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The first Spanish records of *Melanoleuca galbuserae* are published from two localities in the Pyrenees; a third locality published here is situated in the adjacent French part of these mountains. The identity of this species is confirmed morphologically and with phylogenetic methods. Its distribution and ecology are discussed. *Melanoleuca galbuserae* is currently known from France, Italy and Spain and represents a montane to alpine fungus widely distributed in the montane-alpine zone of Mediterranean countries. An analysis of DNA sequence databases indicates occurrence in montane-alpine areas of Asia and also North America. A study of macro- and micromorphological features of these collections also provides new information about the variability of this species.

Key words: *Agaricales*, montane-alpine distribution, Pyrenees, variability, phylogeny.

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Antonín V., Jančovičová S., Ballester L., Caboň M., Ďuriška O., Tomšovský M. (2024): První nálezy tmavobělky Galbuserovy (*Melanoleuca galbuserae*, *Melanoleucaceae*, *Basidiomycota*) ve Španělsku a poznámky k její ekologii. – Czech Mycol. 76(2): 125–138.

V článku jsou publikovány první nálezy tmavobělky Galbuserovy ze Španělska pocházející ze dvou lokalit v Pyrenejích; třetí zde publikovaná lokalita pochází z francouzské části tohoto pohoří. Určení druhu bylo potvrzeno makro- a mikromorfologicky i fylogenetickými metodami. Je diskutováno

jeho rozšíření a ekologie. V současnosti je druh znám z Itálie, Francie a Španělska a představuje horský až alpinský druh rozšířený v oblasti Středozemí. Průzkum veřejně dostupných databází sekvencí DNA naznačuje rozšíření druhu také v horských oblastech Asie a Severní Ameriky. Studium nalezených plodnic přináší též nové a důležité informace o variabilitě morfologických znaků.

INTRODUCTION

The genus *Melanoleuca* Pat. is a taxonomically complicated genus of agarics with recently 35 described European species confirmed (Antonín et al. 2014, 2015, 2017, 2021, 2022, 2023, Ďuriška et al. 2017). It was traditionally classified in *Tricholomataceae* R. Heim ex Pouzar (Pouzar 1983, Singer 1986), but was later included in *Pluteaceae* Kotl. et Pouzar based on a DNA sequence analysis (Matheny et al. 2006, Garnica et al. 2007), while He et al. (2019) considered it *incertae sedis* within *Agaricales*. Today, it belongs to the recently proposed family *Melanoleucaceae* Locq. ex Vizzini, Consiglio et P. Alvarado (Vizzini et al. 2024).

Melanoleuca galbuserae Antonín, Ševcíková, Para et Tomšovský belongs to subgen. *Urticocystis* Boekhout characterised by the presence of urticoid cheilocystidia or the absence of any cheilocystidia. *Melanoleuca galbuserae* is characterised by rather small basidiomata, a light beige to dirty ochre-brown pileus with a darker centre, white, then dirty whitish lamellae, a cylindrical, ochre- to grey-brown stipe, mostly whitish context, (7.2)7.5–9.5(10) × 5.0–6.5(7.0) µm large basidiospores, urticoid cheilocystidia of the *brevipes*- and *exscissa*-type, a pileipellis in the form of a (sub)trichoderm and the absence of a caulohymenium (Antonín et al. 2021). It belongs to the *M. exscissa* (Fr.) Singer group (Antonín et al. 2017). It was originally described from three localities in northern Italy [Trentino-Alto Adige/South Tyrol: Solda (two localities) and Pozza di Fassa; Antonín et al. 2021]. Two years later, two other records of the species were published from the French Alps in Savoie (Armada et al. 2023).

To date, *Melanoleuca galbuserae* has been recorded from three localities in the Pyrenees. Two of them are reported from the Spanish part of these mountains as the first records for Spain, and one from the French part.

MATERIAL AND METHODS

This study is based on collections of fresh basidiomata sampled during field surveys in France and Spain by the second author (S.J.). Macroscopic descriptions of fresh basidiomata are provided. Colour abbreviations follow Kornerup et Wanscher (1983). Authors of fungal names are cited according to the Authors of Fungal Names web page (http://www.indexfungorum.org/AuthorsOfFungalNames.htm). Microscopic features are described from dried material mounted in KOH, Melzer's reagent, and Congo Red, using a Nikon Eclipse Ni (Japan) and an Olympus BX-50 (Japan) light microscope with magnifications of 400× and 1000×. The microscopic description is based on 30 measurements of

Tab. 1. Sequenced specimens of *Melanoleuca* species analysed in the study. The newly obtained sequences are marked in bold.

Species	Country, locality	Herbarium specimen	GenBank acc. no. (ITS)
M. galbuserae	Italy, Trentino-Alto Adige, Pozza di Fassa	MCVE 4505, E. Bizio 1994-08-06	JF908351
M. galbuserae	Italy, Trentino-Alto Adige, Solda	BRNM 825710	MW491333
M. galbuserae	Italy, Trentino-Alto Adige, Solda	BRNM 825709, holotype	MW491332
M. galbuserae	**Spain, Aísa, Rioseta**	**SLO 2755**	**PP931036**
M. galbuserae	**Spain, Canfranc, Rioseta**	**SLO 2776**	**PP931035**
M. galbuserae	**France, Laruns, Col du Pourtalet**	**SLO 2783**	**PP931034**
M. galbuserae	France, Savoie, Peisey-Nancroix	FA 4323	OR419938
M. galbuserae	France, Savoie, Peisey-Nancroix	FA 2083	OR419939
M. galbuserae	USA, Arizona, Cochise County	iNaturalist # 131143582	OP784309
M. grammopodia	Czech Republic, Třemošnice	BRNM 762047	KT279047
M. grammopodia	Slovakia, Veľká Fatra Mts, Vrchlúky	SLO 1463	KP192264
M. grammopodia	Slovakia, Liptovské Revúce	SLO 1468	KP192267
M. grammopodia	Slovakia, Liptovské Revúce	SLO 1466	KP192269
M. griseobrunnea	South Korea, Taean Peninsula, Deoksung	BRNM 781058	LT594152
M. juliannae var. *decolorans*	Italy, Altino di Montemonaco	BRNM 751960, holotype	KJ425532
M. juliannae var. *juliannae*	Hungary, Budapest, Rákospalota	BP 104371, holotype	KJ425539
M. porphyropoda	China	HMAS 267624, holotype	KF220640
M. stepposa	Czech Republic, Ivančice	BRNM 781064	LT594150
M. stepposa	Czech Republic, Brno	BRNM 781099	LT594147
M. tristis	Czech Republic, Třeboň	BRNM 772197	LT594137
M. tristis	Italy, Ravenna, Pineta di S. Vitale	BRNM 772192	LT594135
M. tristis	Slovakia, Lakšárska Nová Ves	SLO 1607	LT594139
M. tristis	Slovakia, Šaštín	SLO 1671	LT594140
M. zaaminensis	Uzbekistan, Pamiro-Alai Mts, Kulsai	TAAM 121360, holotype	LT594141
Melanoleuca sp. Czechia	Czech Republic, Ivančice	BRNM 781065	LT594142
Melanoleuca sp. Korea	South Korea, Mongsanpo	BRNM 781059	LT594153

basidiospores, and 10 measurements of basidia and cystidia of each specimen. For basidiospores, the factors E (quotient of spore length and width) and Q (mean of E-values) are used. For lamellae, L is the number of entire lamellae, and l is the number of lamellulae tiers between each pair of entire lamellae. Characters of cheilocystidia are defined according to Vizzini et al. (2011). The caulohymenium, formed especially on the stipe apex surface in some *Melanoleuca* species, is a layer composed of sporulating caulobasidia, caulobasidioles and caulocystidia. The structures are analogous

to the hymenium of the hymenophore (Šutara 2005). Scanning electron microphotographs of basidiospores were taken using scanning electron microscopy (SEM) device Vega 4 LMU (TESCAN, Brno, Czech Republic). The microscopic structures were coated with a 10 nm gold layer using a Luxor Gold coater (LUXOR Tech, Nazareth, Belgium) before microscopy. The specimens are preserved in the SLO herbarium (herbarium of the Comenius University Bratislava, Slovakia).Total genomic DNA was extracted from dried basidiomata using E.Z.N.A Fungal DNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's recommendations with prolonged incubation time as described in Caboň et al. (2019). Nuclear ribosomal ITS region was amplified with ITS5, ITS4 primers (White et al. 1990) using 5× HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia) applying PCR conditions by Caboň et al. (2019). The PCR products were purified using Exo-Sap enzymes (Thermo Fisher Scientific, Wilmington, Germany) following the manufacturer's recommendations and sequenced at the SeqMe sequencing company (Dobříš, Czech Republic).

Newly generated sequences were aligned to the dataset published in Antonín et al. (2023). Additionally, we searched for other *M. galbuserae* sequences in public databases (GenBank and UNITE) with the type sequence as the query input. In order to understand the distribution of our target species, the type sequence of *M. galbuserae* was also subjected to a BLAST search within the GlobalFungi metabarcoding database (Větrovský et al. 2020). The ITS dataset of *M. galbuserae* and related species was completed with those from GenBank (OR419938 and OR419939 from Savoie, France and OP784309.1 from Arizona, USA). Due to an incomplete length of ITS regions retrieved from the GlobalFungi search, we only confirmed a match with type sequences and did not included these sequences into the phylogenetic analysis. The phylogenetic analysis of the aligned dataset of ITS sequences (Tab. 1) was conducted by means of the maximum likelihood algorithm carried out using Phylogeny.fr (Dereeper et al. 2008), A la Carte mode (Maximum Likelihood method using PhyML, GBlock disabled, GTR evolutionary model, bootstrapping procedure with 100 replicates).

RESULTS

PHYLOGENY

Phylogenetic analysis confirmed conspecificity of the Pyrenean specimens with Italian and French collections of *Melanoleuca galbuserae* (Fig. 1), although some intraspecific variability (3 differences out of 630 nucleotide positions) was revealed. Search for additional *M. galbuserae* ITS sequences deposited in public databases yielded a single sequence from a collection from Chiricahua National Monument (Arizona, USA; iNaturalist ID: 131143582, GenBank: OP784309). Search in the GlobalFungi metabarcoding database resulted in an ITS1 sequence genotype identical to the sequence of *M. galbuserae* holotype, identified in one soil sample collected in Niwot Ridge (Colorado, USA) and two soil samples collected in Qinhai Province, China (Tibetan plateau): Gangcha County and Haibei Research Station. Similar to our finds, all four collections/samples were reported from montane or alpine habitats.

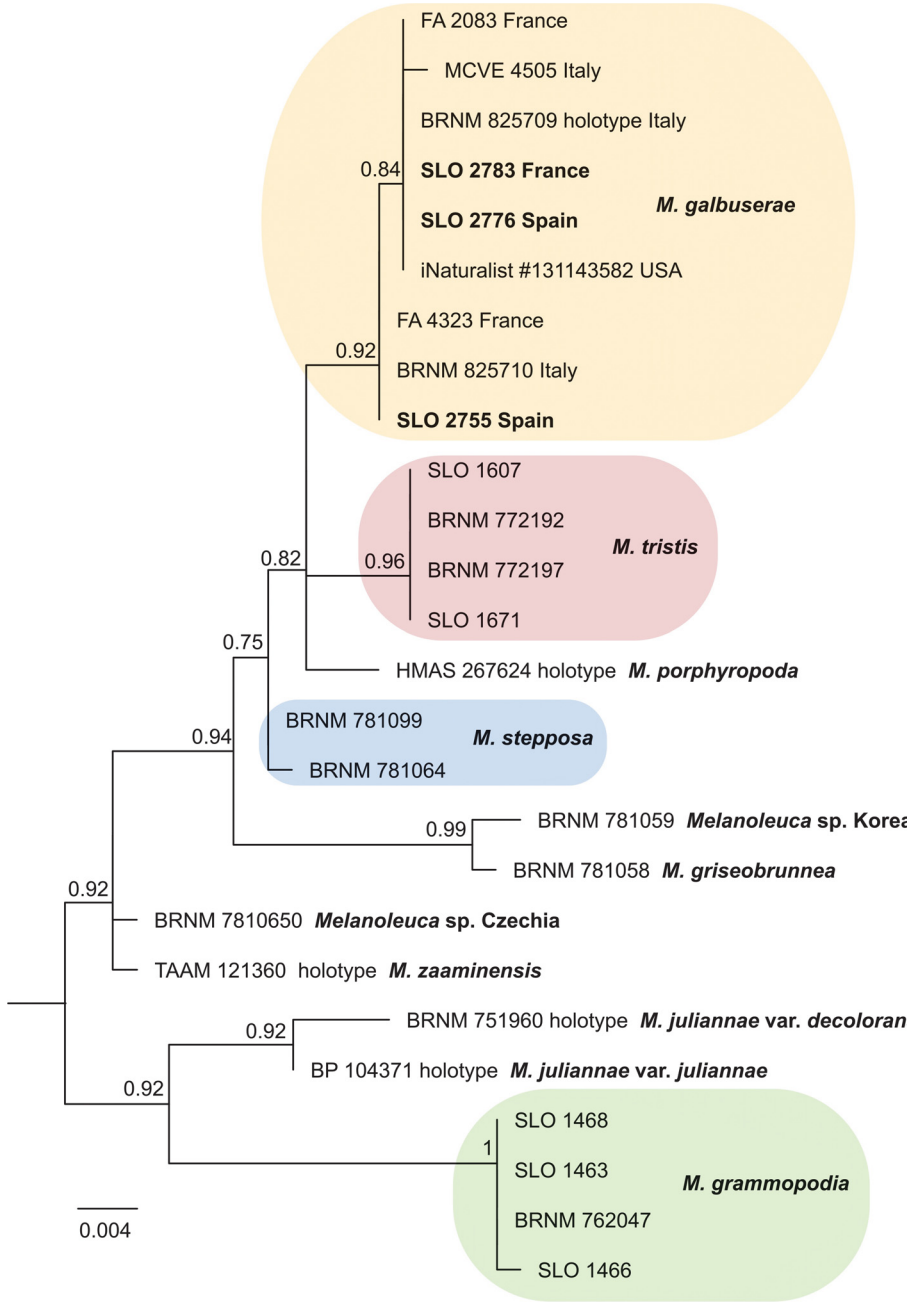

Fig. 1. Phylogenetic tree confirming the identity of Pyrenean specimens with *M. galbuserae*. The newly obtained sequences are highlighted in bold. Numbers at branches indicate maximum likelihood bootstrap support. The bar indicates the number of expected substitutions per position.

Description and ecology

Melanoleuca galbuserae Antonín, Ševčíková, Para et Tomšovský, in Antonín, Ševčíková, Para, Ďuriška, Kudláček et Tomšovský, Journal of Fungi 7(3): 191, p. 8, 2021 Figs 2–7

Collected specimens

Pileus 13–40 mm in diam., convex, plano-convex to applanate, umbonate or depressed in the centre, without central umbo; margin involute, then straight to inflexed, non-striate, surface velutinous, smooth or slightly dimpled in some places, yellowish brown (tobacco – 5F6, sepia – 5F4) to brown (6E4) or chocolate (6F4) in the centre, yellowish brown (hair brown – 5E4, mustard brown – 5E6) to beige (6E3) at the margin. Lamellae close, L = 56–64, l = 1–3, adnate or emarginate, 3–5 mm wide, orange-grey (5B2), greyish brown (nougat – 5D3) to light brown (5D4), edge entire, concolorous. Stipe 10–30 mm long, 3–9 mm broad, broader or narrower at the base (4–7 mm), longitudinally fibrillose, white pruinose at apex, light brown (5D4) to brown (6E4), or yellowish brown (hair brown – 5E4, bronze – 5E5) in the upper part and yellowish brown (mustard brown – 5E6) at the base. Stipe trama orange-white to orange-grey (5A2, 5B2) in the upper half, and greyish orange (5B3), brownish orange (5C3) or light brown (5D4) at the base; smell chemical or fungoid.

Basidiospores 7.2–10 × 4.8–6.8 µm, average = 8.5 × 5.7 µm, E = (1.17) 1.32–1.80, Q = 1.51, broadly ellipsoid to ellipsoid, thin-walled, with verruculose ornamentation, amyloid; ornamentation consisting of isolated obtuse warts with a circular, ellipsoid or less frequently irregular outline. Basidia 16.7–40 × 6.5–12 µm, 4-spored, sometimes 2-spored, clavate, sometimes cylindrical. Basidioles 21–25 × 5.3–7.0 µm, clavate. Cheilocystidia 20–43 × (2.5)4.0–8.0 µm, scattered, urticoid, mainly of both the *brevipes*- and *exscissa*-type, apical part 1–2.5 µm, thin-walled, with one septum or rarely without a septum, most frequently incrusted, rarely not incrusted at the apex. Pleurocystidia (20)26–37 × (3.6)5.5–7.6 µm, very rare, urticoid, of the *brevipes*- and *exscissa*-type, apical part 1–2.5 µm, thin-walled, without septum or with one septum, both non-incrusted or incrusted at the apex. Pileipellis a cutis composed of cylindrical, thin-walled, 3.0–8.7 µm wide hyphae. Stipitipellis composed of cylindrical, parallel, ± thin-walled, 2.0–9.0 µm wide, sometimes minutely brown incrusted hyphae. Caulohymenium present. Caulocystidia scattered, of two types: (1) 20–63 × 2.2–5.0 µm, cylindrical, thin-walled, with or without a septum, not incrusted at the apex; (2) urticoid (*brevipes*-type), basal part 30–52 × 4.0–5.5 µm, apical part 2.0–2.5 µm thick, thin-walled, with one septum, not incrusted at the apex. Caulobasidium (one observed) 23 × 7.2 µm, 2-spored, clavate, not observed in most collections. Clamp connections absent.

Fig. 2. *Melanoleuca galbuserae* basidiomata collected in Spain, Pyrenees, municipality of Aísa, Rioseta, 3 October 2022, SLO 2755.
Photo S. Jančovičová.

Fig. 3. *Melanoleuca galbuserae* basidiomata collected in Spain, Pyrenees, municipality of Canfranc, Rioseta, 6 October 2022, SLO 2776.
Photo S. Jančovičová.

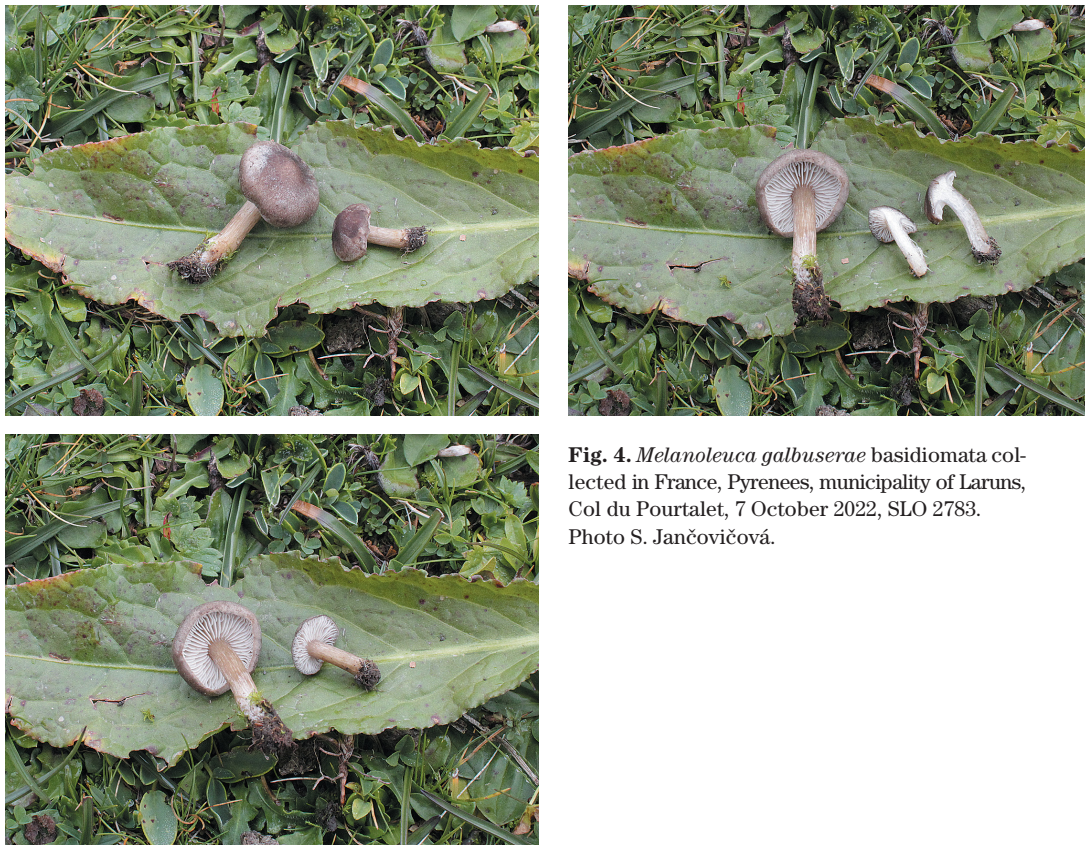

Fig. 4. *Melanoleuca galbuserae* basidiomata collected in France, Pyrenees, municipality of Laruns, Col du Pourtalet, 7 October 2022, SLO 2783. Photo S. Jančovičová.

Collections studied

France. Pyrenees, municipality of Laruns, Col du Pourtalet, NW exposure, 42°49'01.20" N, 00°24'57.24" W, 1730–1770 m a.s.l., 7 October 2022, leg. S. Jančovičová (SLO 2783).

Spain. Pyrenees, municipality of Aísa, Rioseta, NW exposure, 42°46'26.04" N, 00°30'56.16" W, 1400–1500 m a.s.l., 3 October 2022, leg. S. Jančovičová (SLO 2755). – Pyrenees, municipality of Canfranc, Rioseta, NW exposure, 42°46'14.52" N, 00°30'58.32" W, 1400–1500 m a.s.l., 6 October 2022, leg. S. Jančovičová (SLO 2776).

Ecology

Melanoleuca galbuserae was collected at two Spanish localities in an area of the Aragon Valley called Rioseta (municipalities of Aísa and Canfranc) in the Pyrenees. It grew here at an elevation of 1400–1500 m a.s.l. in mountain pastures with scattered *Pinus sylvestris*, *Buxus sempervirens*, and *Rosa canina*, and an undergrowth vegetation of *Anthriscus sylvestris*, *Cuscuta europaea*, *Cirsium*

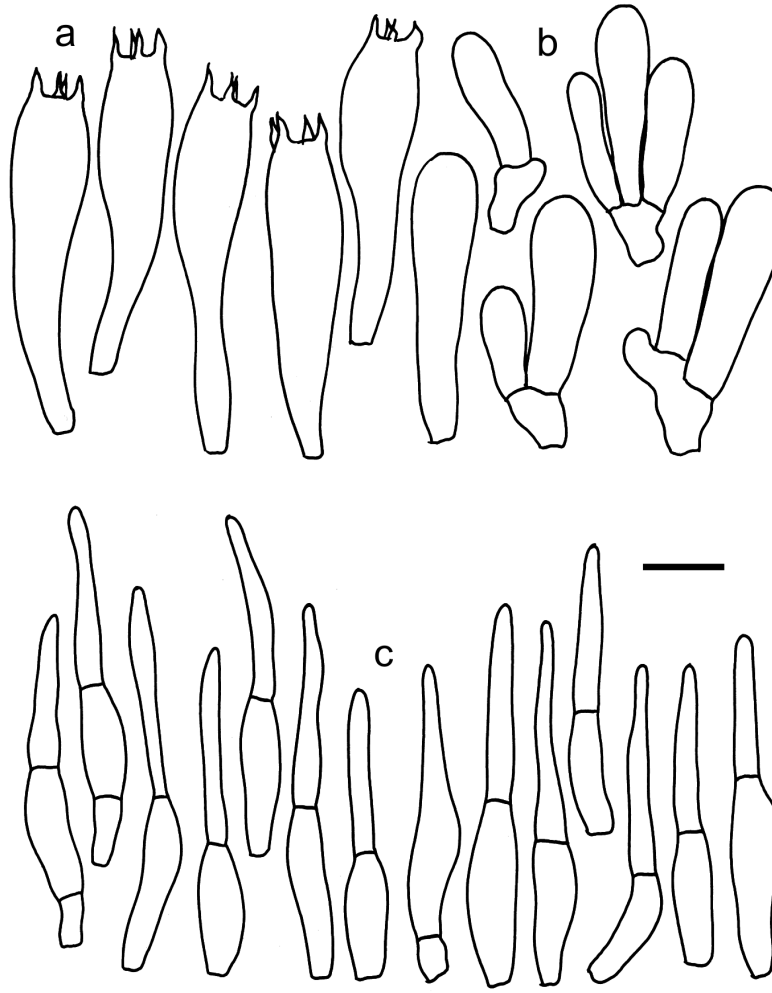

Fig. 5. *Melanoleuca galbuserae*, SLO 2783 (for details, see Collection studied): **a** – basidia, **b** – basidioles, **c** – cheilocystidia. Scale bar = 10 μm. Drawing S. Jančovičová.

eriophorum, *Centaurea nigra*, *Cynoglossum officinale*, *C. germanicum*, *Dittrichia graveolens*, *Draba dubia*, *Echinospartum horridum*, *Galium pumilum*, *Hyoscyamus niger*, *Polygala calcarea*, *Polypodium vulgare*, and *Saponaria officinalis*. At Col du Pourtalet (municipality of Laruns) in the French Pyrenees at an elevation of 1730–1770 m a.s.l., the species also grew in montane pastures, but in an alpine plant community with *Dryas octopetala*, *Salix herbacea*, *S. pyrenaica*, *S. reticulata*, *Dianthus benearnensis*, *Gentiana lutea*, *Iris latifolia*, *Nardus stricta*, *Trifolium alpinum*, *Aconitum vulparia*, *Allium senescens*,

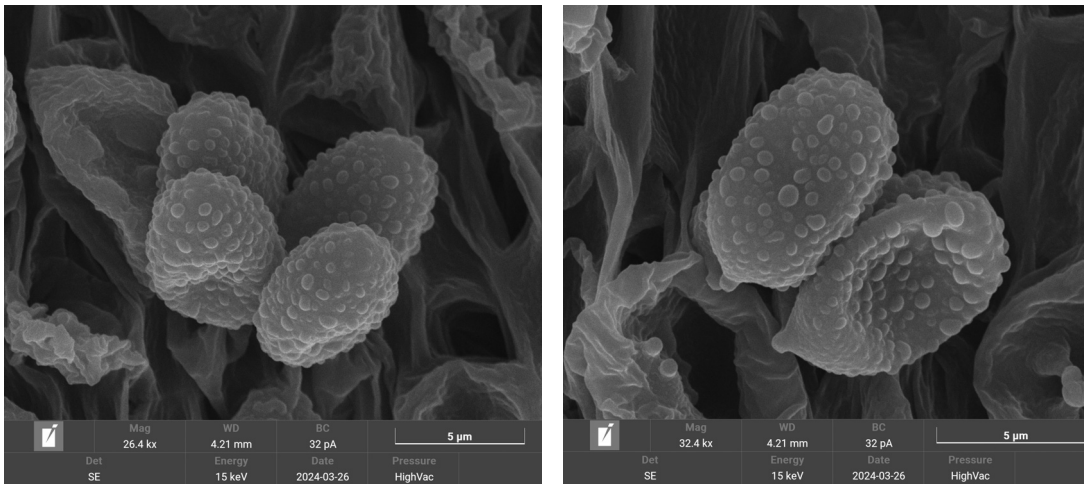

Fig. 6. *Melanoleuca galbuserae*, SLO 2783 (for details, see Collection studied): basidiospores under scanning electron microscope. Photo R. Plichta and M. Tomšovský.

Cotoneaster integerrimus, *Thalictrum minus*, and *Vaccinium myrtillus*. The ecological preferences of *M. galbuserae* for montane/alpine grasslands is also supported by additional sequences retrieved from public databases. Occurrence in the USA is reported from montane areas of Arizona and Colorado, while both occurrences in China are reported from alpine grasslands.

Distribution

Melanoleuca galbuserae is currently known from France (Savoyan Alps and Pyrenees; Armada et al. 2023 and this study), Italy (Trentino-Alto Adige region; Antonín et al. 2021) and Spain (Pyrenees; this study). Sequences deposited in public databases suggest a wider distribution across the Northern Hemisphere with two records from the USA and two records from China. Even though these sequences are identical to the sequence of the type, further studies based on multi-loci analysis are needed to confirm if these reports represent identical species.

DISCUSSION

The additional collections of *Melanoleuca galbuserae* presented in this contribution not only extend the known distribution range of this species, but also provide new information about the variability of its macro- and microscopic characters.

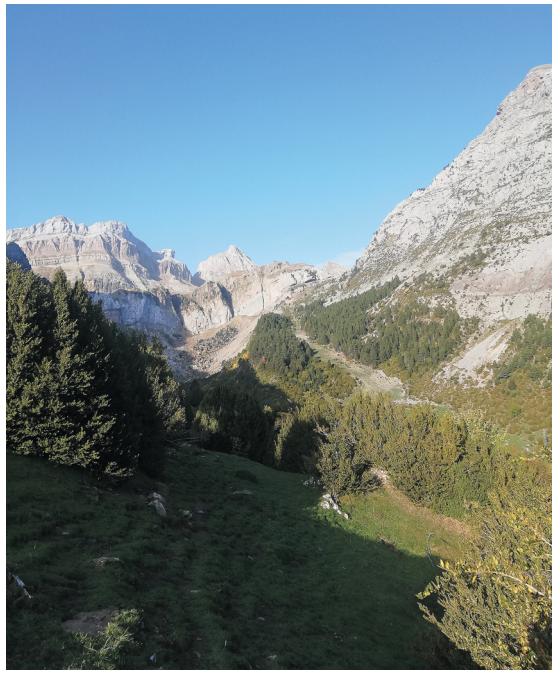

Fig. 7. *Melanoleuca galbuserae* localities: **above** – France, Pyrenees, municipality of Laruns, Col du Pourtalet, 7 October 2022; **below** – Spain, Pyrenees, municipality of Canfranc, Rioseta, 6 October 2022. Photo S. Jančovičová.

Macroscopically, (1) lamellae may also be orange-grey, greyish brown to light brown, and (2) the context of the stipe base (originally described as white to greyish) can also be light brown, greyish orange or brownish orange. Microscopically, the collections reported here agree with the original description in most of the features but add several characters to the variability known so far: (1) the basidiospore ornamentation may be composed of only warts (lacking small and sometimes scarce ridges), (2) the apical part of the cheilocystidia can also be just 1–2.5 µm wide, (3) very rarely, pleurocystidia similar to cheilocystidia can also be present, (4) the pileipellis may also be in the form of a cutis, and (5) the caulohymenium can also be developed, but caulobasidia are extremely rare. Using the identification key for European species of the subgen. *Urticocystis* (Antonín et al. 2023), two new features can lead to misidentifications: (1) the (extremely) rare presence of pleurocystidia, and (2) the colour of the context of the stipe base (light brown, 5D4) of specimen SLO 2783. *Melanoleuca galbuserae* is placed among species with a stipe base context coloured white or whitish, rarely pale grey whitish, brown whitish or with a fine orange-brown tinge in this key. However, a distinctly darker (brown, black-brown, yellowish brown, dark grey, etc.) context never appears in *M. galbuserae*.

The localities published in the present contribution distinctly expand the distribution range of *M. galbuserae*. The species is described from northern Italy (Trentino-Alto Adige), and was later recorded in the Savoyan Alps in eastern France (Armada et al. 2023). At present the boundaries of the distribution range extend to the Pyrenees. The records of *M. galbuserae* from North America and Asia are very surprising, although those also refer to a montane habitat. A geographic barrier usually leads to a speciation process, so that a detailed study including samples from various areas of the Northern Hemisphere would be desirable.

Melanoleuca galbuserae was originally collected in alpine grasslands and pastures with *Salix herbacea* (Antonín et al. 2021) at elevations of 2530–2820 m. Similar habitats with alpine dwarf *Salix* spp. and other alpine plant species (e.g. *Dryas octopetala*) at an elevation of 2050–2500 m were published by Armada et al. (2023) and resemble that of collection SLO 2783 from the French Pyrenees (elevation 1730–1770 m, see above). Nevertheless, Spanish Pyrenean collections were recorded in different habitats, i.e. mountain pastures at an elevation of 1400–1500 m, with scattered *Pinus sylvestris*, *Buxus sempervirens*, *Rosa canina* and other, typical montane plants (see above). These localities prove that the species is not restricted to the alpine zone, but can also descend to the montane zone close to the treeline.

In the same habitat as the Spanish collections of *M. galbuserae*, two other *Melanoleuca* species were found: *M. friesii* (Bres.) Bon (municipality of Aísa, 3 October 2022, SLO 2756), and *M. exscissa* (Fr.: Fr.) Singer (municipality of Canfranc, 6 October 2022, SLO 2777); for complete details, see Collections studied.

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