Melanoleuca monticola and M. romanensis, two new European species of Melanoleuca, and comments to M. graminicola group

Vladimír Antonín (vantonin@mzm.cz)
Moravian Museum

Ondrej Ďuriška
Comenius University in Bratislava

Soňa Jančovičová
Comenius University in Bratislava

Tomáš Kudláček
Mendel University in Brno

Roberto Para
Via Martiri di via Fani 22

Hana Ševčíková
Moravian Museum

Michal Tomšovský
Mendel University in Brno

Research Article

Keywords: Agaricomycetes, subg. Urticocystis, ITS, rpb2, tef1, systematics

Posted Date: January 3rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2404494/v1

Abstract

Melanoleuca is one of the taxonomically most complicated genera of Agaricomycetes. The aim of this study is taxonomic revision of European specimens confused with *M. angelesiana* A.H. Sm. and delimitation of four species, *M. brachyspora* Harmaja, *M. graminicola* (Velen.) Kühner & Maire, *M. rufipes* Bon and *M. stridula* (Fr.) Singer. The research is based on macro- and micromorphological characters and the multigene phylogenetic analyses of a combined (ITS, rpb2, and tef1) dataset. According to the result, the two new species are proposed – *M. monticola* sp. nov. which accomodates European specimens previously confused with American species *M. angelesiana* and *M. romanensis* sp. nov. from Italy. Moreover, American specimens identified as *M. angelesiana* belongs to three different phylogenetic species (*M. angelesiana* sensu typi, *M. acystidiata* aff. and one more separate lineage). *M. graminicola* is emended and the respective epitype designated. An identification key of the European species of subg. *Urticocystis* is provided.

Highlights

- Description of two new species of the agaricoid genus *Melanoleuca* from Europe.
- Multigene analysis based on a combined three genes dataset.
- A key to the identification of the European species of *Melanoleuca*, subg. *Urticocystis*.

Introduction

Melanoleuca Pat. is a morphologically well-defined genus of agaricomycetous fungi (Agaricales, incertae sedis; He at al. 2019), characterized by colourless basidiospores with amyloid ornamentation and the absence of clamp connections (e.g. Boekhout 1988; Bon 1991; Vizzini et al. 2011 Vesterholt 2012). Anyway, the most *Melanoleuca* species have the substantial morphological variability and macro- or micromorphological characters are overlapping in many cases, therefore these characters are not sufficient for identification at species level. The using of DNA sequences may be necessary for identification of some problematic taxa (e.g., Vizzini et al. 2011; Yu et al. 2014; Antonín et al. 2014, 2015, 2017, 2021). The genus currently includes over 440 validly published names (http://www.indexfungorum.org, accessed on 3 June 2022) and around 60 species globally accepted (He et al. 2019).

In the past, different concepts of intrageneric classifications were published (e. g. Métrod 1948; Singer 1986; Kühner 1978; Boekhout 1988, 1999; Bon 1991). Recently, two subgenera within the genus are recognized, subg. *Melanoleuca* emend. Fontenla, Para & Vizzini characterized by macrocystidia in hymenium, and subg. *Urticocystis* Boekhout including species with urticocystidia or completely lacking any cystidia in hymenium (Vizzini et al. 2011).

Since 2012, *Melanoleuca* has been a subject of extensive taxonomical revision, focused on European taxa (Antonín et al. 2014, 2015, 2017, 2018, 2021, 2022; Ďuriška et al. 2017). Antonín et al. (2022) revised

European species of the subg. *Melanoleuca* and delimited 11 species within the subgenus: *M. albomarginata* Antonín, Ďuriška, Jančovičová, Para & Tomšovský, *M. ammophila* Antonín, Ďuriška, Jančovičová, Para & Tomšovský, *M. bataillei* Malençon, *M. cavipes* Métrod ex Bon, *M. friesii* (Bres.) Bon, *M. granadensis* Armada, *M. melaleuca* (Pers.) Murrill, *M. pallidicutis* Bresinsky, *M. polioleuca* (Fr.) Kühner & Maire, *M. strictipes* (P. Karst.) Jul. Schäff. and *M. variabilis* Antonín, Ďuriška, Jančovičová, Para & Tomšovský.

Taxonomic revision of European species of the subg. *Urticocystis* was subject of series of studies (Antonín et al. 2014, 2015, 2017, 2021). Till now, 20 taxa of the subgenus were recognized and delimited: *M. acystidiata* Para, Antonín, Ševčíková, Ďuriška & Tomšovský, *M. castaneofusca* Contu, *M. cognata* (Fr.) Konrad & Maubl., *M. diverticulata* G. Moreno & Bon, *M. exscissa* (Fr.: Fr.) Singer, *M. fontenlae* Para, Antonín, Ďuriška, Ševčíková & Tomšovský, *M. galbuserae* Antonín, Ševčíková, Para & Tomšovský, *M. grammopodia* (Bull. : Fr.) Pat., *M. humilis* (Pers.) Pat., *M. juliannae* Rimóczi, Antonín, L. Nagy & Tomšovský and *M. juliannae* var. *decolorans* Antonín & Tomšovský, *M. luteolosperma* (Britzelm.) Singer, *M. malenconii* Bon (Antonín et al. 2015), M. *microcephala* (P. Karst.) Singer, *M. paedida* (Fr.) Kühner & Maire, *M. rasilis* (Fr.) Singer, *M. stepposa* Vacek, *M. stridula* (Fr.) Singer, *M. tristis* M.M. Moser, and *M. verrucipes* (Fr.) Singer.

This work continues in the taxonomical revisions focused on an European taxon confused with *M. angelesiana* A.H. Sm. and delimitation of four species, *M. brachyspora* Harmaja, *M. graminicola* (Velen.) Kühner & Maire, *M. rufipes* Bon and *M. stridula* (Fr.) Singer. Delimitation of the remaining species formally encloses taxonomical revision of the genus *Melanoleuca* in Europe. A key to identification of the European species of subg. *Urticocystis* is published.

Material And Methods

Morphological analyses

This study is based on collections of fresh basidiomata made during field surveys in Europe deposited in various herbaria, especially ANC, BRNM and SLO. Six *Melanoleuca* type specimens preserved in the following herbaria were included in this study: ANC [*Melanoleuca stridula* (Fr.) Singer], H [*M. brachyspora* Harmaja], LIP [*M. rufipes* Bon], MPU (*M. electropoda* Maire & Malençon), PC (*M. striimarginata* Métrod), PRM [*M. graminicola* (Velen.) Maire & Kühner]. Macroscopic descriptions of collected specimens are given of fresh basidiomata. Colour abbreviations follow Kornerup and Wanscher (1983) and Küppers (2007), and herbarium abbreviations follow Thiers (2022). 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 an Olympus BX-50 light microscope (Japan) with a magnification of 400× and 1000×. Finger test (Bon 1991) means that the finger print is visible at the pileus surface after touching. For basidiospores, the factors E (quotient of length and width in any one spore) 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 caulobasidioles, caulocystidia and sporulating caulobasidia. It is comparable with the hymenium of the hymenophore in many respects (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 the microscopy.

Dna Sequence Dataset

DNA from dried fungal material was isolated and three genes (ITS region of ribosomal RNA gene = ITS; RNA polymerase II, the second largest subunit = rpb2; translation elongation factor 1-alpha = tef1) were amplified according to Antonín et al. (2015, 2017). In the case of older type specimens the genus-specific primers for the Melanoleuca-targeting ITS2 region (MELITS2F/MELITS2R) developed by Antonín et al. (2015) were applied for amplification. The two datasets were the subject of phylogenetic analyses. The ITS-only dataset contained of all specimens including the type specimens whereas the combined multilocus ITS- rpb2-tef1 dataset contained selected representatives of each species. The sequences were aligned using MAFFT, version 7 online program, setting up the Q-INS-i option (Katoh and Toh 2008).

The datasets were enriched with sequences published mostly by Vizzini et al. (2011), Osmundson et al. (2013), Sánchez-García et al. (2013), Yu et al. (2014), Antonín et al. (2014, 2015, 2017). The aligned ITS dataset was 785 bp long and included 484 conserved, 286 variable, and 36 singleton positions as determined in the MEGA X program version 10.1.8 (Kumar et al. 2018). The combined ITS- rpb2-tef1 dataset was 2751 bp long (1645 conserved, 1073 variable, and 142 singleton positions). The DNA sequences of two-three species from subg. *Melanoleuca* were selected as the outgroup for both ITS only and multilocus datasets.

Phylogenetic Analysis

Both datasets were subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses.

For each gene best-fit partitioning scheme was found with PartitionFinder 2 (Lanfear et al. 2016) based on codon positions as data blocks using the corrected Akaike Information Criterion (AICc). For both datasets, the analysis was performed for both linked and unlinked branch lengths with the results being exactly the same in both cases. All possible partitioning schemes were analysed (the option search = all;). The resulting partitioning schemes were as follows: A) ITS — all three codon positions were merged into one partition. B) rpb2 — each codon position was selected as a separate partition. C) tef1 — the first

codon position was selected as a separate partition, the second and third positions together formed another partition.

ML phylogenetic inference was performed with RAxML-NG 1.1.0 (Kozlov et al. 2019). The best-fitting evolutionary model for each partition was selected by PartitionFinder 2 (Lanfear et al. 2016). The set of all 84 available models was used that included also models with base frequencies estimated by ML (the option models = allx;). The number of bootstraps was determined using the MRE-based bootstopping test (Pattengale et al. 2010). The cutoff value was set to 0.01 (the option –bs-cutoff 0.01). As a branch support Transfer Bootstrap Expectation metric (Lemoine et al. 2018) was computed. The support values were mapped onto the best-scoring tree.

BI was carried out using BEAST 2 (Bouckaert et al. 2014). In both analyses the uncorrelated log-normal relaxed molecular clock was used (Drummond et al. 2006). Substitution models for individual partitions were selected automatically via model averaging implemented in the bModelTest package (Bouckaert and Drummond 2017). For both analyses Metropolis coupled MCMC (MC3) was applied using CoupledMCMC package (Müller and Bouckaert 2019). Four chains were used – three heated and one cold. The chain length was always set to 20,000. 000 and every 5000th generation was sampled. Target switch probability was set to 0.234 (Kone and Kofke 2005; Atchadé et al. 2011) and the 25% burn-in was used. The posterior parameter estimates were summarised using Tracer 1.7.1 (Rambaut et al. 2018). Quality of the estimates was assessed based on visual analysis of the trace plots and ESS values – the ESS value ≥ 200 indicated proper sampling (standard approach). Parameter estimates were summarized with TreeAnnotator 2.6.0 (part of BEAST 2) and mapped onto the 50% majority-rule consensus tree created with SumTrees 4.4.0 (Sukumaran and Holder 2010). Edge lengths were calculated as mean lengths for the corresponding edges in the input set of trees.

Results

Phylogeny

Phylogenetic analyses confirmed separate position of previously recognised species (Antonín et al. 2014, 2015, 2017, 2021). Moreover, the position of *M. brachyspora* and *M. graminicola* within subg. *Urticocystis* excluded from the previous research is clarified (Figs. 1 and 2). The multigene analyses confirmed the structure of the two main *Melanoleuca* species groups named in previous papers (Antonín et al. 2017, 2021) as the *M. exscissa* and the *M. castaneofusca* groups. The position of apomorphic *M. cognata* is closer to the *M. castaneofusca* group.

The separation of some species (*M. stridula*, *M. angelesiana* aff. USA — see details below) is not statistically supported by analyses of the ITS-only dataset (Fig. 1), but are well delimited by the multilocus data analyses.

Table 1
The sequenced specimens analysed in the study. The newly obtained sequences are marked in bold.

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. acystidiata	Italy, South Tyrol, St. Jacob in Val di Vizze	ANC M0205, holotype	JN616462		
M. acystidiata	Switzerland, Davos, Schatzalp	BRNM 772203	MW491319	MW488169	MW488154
M. acystidiata aff. USA	USA, Colorado, Front Range	DBG 28118	**OP394180**		
M. acystidiata aff. USA	USA, Colorado, Caribou trail head, Roosevelt NF	DGB 28732	**OP394181**		
M. acystidiata aff. USA	USA, Colorado, Caribou creek	DGB 39834	**OP394182**	**OP454885**	
M. angelesiana	USA, Washington, Olympic Mts., Lake Angels	AFS 11438, syntype	MW491318		
M. angelesiana aff. USA	USA, Idaho, Payette Nat. Forest, near Brundage Reservoir	NY 1772313, OKM26930	**OP394183**	**OP454886**	
M. angelesiana aff. USA	USA, Idaho, Payette Nat. Forest, Brundage Reservoir Road	NY 1927565, OKM26963	**OP394184**	**OP454887**	
M. brachyspora	Finland, Varsinais-Suomi, Karkkila, Haavisto	H 6003414, holotype	**OP394185**		
M. brachyspora	Czech Republic, Novohradské hory Mts., Malonty	BRNM 829070	**OP394186**		
M. brachyspora	Slovakia, Vysoké Tatry Mts., Podbanské	SLO 1565	**OP394189**		
M. brachyspora	Czech Republic, České Švýcarsko National Park, Doubice	BRNM 772200	**OP394187**	**OP454888**	**OP454872**
M. brachyspora	Slovakia, Veporské vrchy Mts., Hriňová, Biele Vody	BRNM 829071	**OP394188**	**OP454889**	**OP454873**

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. brachyspora	Slovakia, Vysoké Tatry Mts., Podbanské	SLO 1552	**OP394190**	**OP454890**	**OP454874**
M. brachyspora	Turkey	GB65588	MG989685		
M. castaneofusca	Italy, Ravenna, Pineta di S. Vitale	BRNM 761900	MW491323	MW488170	MW488155
M. castaneofusca	Czech Republic, Hořovice, Osek	BRNM 761901	MW491320	MW488171	MW488156
M. castaneofusca	UK, England, Surrey, Kew, Royal Botanic Gardens	K(M)92562	MW491321	MW488172	MW488157
M. cognata	Sweden, Västergötland, Trollhättan	GB65454	JX429190		
M. cognata	Czech Republic, Toulovcovy maštale Nature Reserve	BRNM 699542	**OP394191**	**OP454891**	**OP454875**
M. diverticulata	Slovakia, Bratislava, Lamač	SLO 1566	LT594155	LT594188	LT594172
M. exscissa	Hungary, Bátorliget	BRNM 772198	LT594125	LT594189	LT594173
M. exscissa	Czech Republic, Mokrsko	BRNM 781061	LT594122	LT594191	LT594175
M. exscissa	Italy, Ravenna, Pineta di S. Vitale	BRNM 781066	LT594123	LT594190	LT594174
M. fontenlae	Italy, Ravenna district, Lido di Dante, holotype	BRNM 772194, holotype	MW491326	MW488173	MW488158
M. fontenlae	Slovakia, Cerová vrchovina Mts., Vlčia dolina	SAV F-3823	MW491327	MW488174	MW488159
M. friesii	Slovakia	BRNM 817799	MT270866	MT268606	MT268581
M. galbuserae	Italy, Trentino, Pozza di Fassa	MCVE4505, E. Bizio 1994-08-06	JF908351	MW488175	MW488160
M. galbuserae	Italy, South Tyrol, Solda	BRNM 825710	MW491333	MW488176	MW488161

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. graminicola	Italy		JN616438		
M. graminicola	Czech Republic, Toulovcovy maštale Nature Reserve	BRNM 829064, epitype	**OP394192**		
M. graminicola	Slovakia, Strážovské vrchy Mts., Pružina-Priedhorie	BRNM 829062	**OP394193**		
M. graminicola	Slovakia, Západné Beskydy, Oravská polhora, Slaná voda	SLO 1523	**OP394194**	**OP454892**	**OP454876**
M. graminicola	Slovakia, Západné Beskydy, Mútne, Mútňanská píla	SLO 1532	**OP394195**	**OP454893**	**OP454877**
M. graminicola	Slovakia, Veľká Fatra Mts, Liptovské Revúce, Zelená dolina	SLO 1623	**OP394196**	**OP454895**	**OP454879**
M. graminicola	Slovakia, Západné Beskydy, Mútne, Mútňanská píla	SLO 1527	**OP394197**	**OP454894**	**OP454878**
M. graminicola	Sweden, Gotland, Viklau	GB65588	JX429216		
M. grammopodia	Czech Republic, Třemošnice	BRNM 762047	KT279047	KT279059	KT279048
M. grammopodia	Slovakia, Veľká Fatra Mts., Vrchlúky	SLO 1463	KP192264	KT279058	KT279049
M. grammopodia	Slovakia, Liptovské Revúce	SLO 1468	KP192267	KT279061	KT279051
M. grammopodia	Slovakia, Liptovské Revúce	SLO 1466	KP192269	KT279060	KT279050
M. griseobrunnea	South Korea, Taean Peninsula, Deoksung	BRNM 781058	LT594152	LT594179	LT594165
M. humilis	Czech Republic, Přerov	BRNM 751965	KJ425530	KT279057	KJ425543
M. humilis	Czech Republic, Kroměříž	BRNM 710023	KJ425531	KT279055	KJ425544
M. humilis	Czech Republic, České Budějovice	CB	KP192290	KT279056	KT279052

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. juliannae var. *decolorans*	Italy, Altino di Montemonaco	BRNM 751960, holotype	KJ425532	LT594181	KJ425545
M. juliannae var. *juliannae*	Hungary, Budapest, Rákospalota	BP 104371, holotype	KJ425539	LT594182	KJ425552
M. leucopoda	HMAS 267626	KF220638			
M. longisterigma	Mexico, Veracruz	ENCB, Guzmán 19274	JX429211		
M. luteolosperma	Slovakia, Velká Fatra Mts., Lubochňa	BRNM 761907	MW491328	MW488177	MW488162
M. luteolosperma	Czech Republic, Srbsko,	BRNM 817820	MW491329	MW488178	MW488163
M. malenconii	Czech Republic, Roudnice nad Labem	BRNM 762051	KP192275	KT279062	KT279053
M. malenconii	Slovakia, Bratislava	SLO 1455	KP192277	KT279063	KT279054
M. microcephala	Slovakia, Velká Fatra Mts., Ružomberok, Skalná Alpa	BRNM 817787	MW491334	MW488179	MW488164
M. microcephala	Slovakia, Velká Fatra Mts., Ružomberok, Skalná Alpa	BRNM 817788	MW491335	MW488180	MW488165
M. monticola, sp. nov.	Slovakia, Vysoké Tatry Mts., Podbanské	SLO 1556, holotype	**OP394198**	**OP454897**	**OP454881**
M. monticola, sp. nov.	Czech Republic, Pohoří na Šumavě	BRNM 829076	**OP394200**	**OP454896**	**OP454880**
M. monticola, sp. nov.	Slovakia, Veľká Fatra Mts., Vlkolínec	SLO 1597	**OP394201**	**OP454898**	**OP454882**
M. monticola, sp. nov.	Slovakia, Trstená	BRNM 653033	**OP394199**		
M. monticola, sp. nov.	Italy	ANC M0203	JN616420		
M. paedida	Italy, Lombardy, Lago Cancano di Valfurva	R. Para 010907-02	MW491337	MW488181	MW488166
M. paedida	Italy, Calabria, Colamauci di Celico	ANC M0189	JN616452		

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. porphyropoda	China	HMAS 267624, holotype	KF220640		
M. rasilis	Italy, Monti Sibillini National Park	BRNM 751967	LT594154,	LT594187	LT594171
M. romanensis, sp. nov.	Italy, Ravenna, Lido di Dante	BRNM 772193, holotype	**OP394202**	**OP454900**	
M. romanensis, sp. nov.	Italy, Ravenna, Pineta Ramazzotti and dunes di Lido di Dante	BRNM 829059	**OP394203**	**OP454899**	
M. romanensis, sp. nov.	Italy, Ravenna, Pineta di Classe, part Pinarella-Bosconi	BRNM 829060	**OP394204**		
M. romanensis, sp. nov.	Italy, Ravenna, Pineta di Classe, Anello di Dante	BRNM 829061	**OP394205**		
M. stepposa	Czech Republic, Ivančice	BRNM 781064	LT594150	LT594176	LT594162
M. stepposa	Czech Republic, Brno	BRNM 781099	LT594147	LT594177	LT594163
M. strictipes	Czech Republic, Staré Hamry	BRNM 737301	KY417098	MT268613	MT268561
M. stridula	Slovakia, Podskalie	BRNM 825716	MW491340		
M. stridula	Austria, Ehrwald	BRNM 825717	MW491338	MW488182	MW488167
M. stridula	Slovakia, Liptovský Hrádok, Hybe	SLO 1543	MW491339	MW488183	MW488168
M. stridula	Italy	ANC M0007, neotype	JN616467		
M. stridula	Slovakia, Považský Inovec Mts., Bojná	BRNM 772202	**OP394206**	**OP454901**	**OP454883**
M. stridula	France, Hautes Alpes Dept., Arvieux, Lac de Roue	BRNM 829065	**OP394207**		

Species	Country, locality	Herbarium specimen	Genbank acc. No. (ITS)	Genbank acc. No. (rpb2)	Genbank acc. No. (tef1)
M. striimarginata	Italy	ANC M0202	JN616468		
M. tristis	Czech Republic, Třeboň	BRNM 772197	LT594137	LT594184	LT594168
M. tristis	Italy, Ravenna, Pineta di S. Vitale	BRNM 772192	LT594135	LT594183	LT594167
M. tristis	Slovakia, Lakšárska Nová Ves	SLO 1607	LT594139	LT594185	LT594169
M. tristis	Slovakia, Šaštín	SLO 1671	LT594140	LT594186	LT594170
M. verrucipes		AFTOL-ID 818	DQ490642		
M. verrucipes	Czech Republic, Hluboš	BRNM 771972	**OP394208**	**OP454902**	**OP454884**
M. zaaminensis	Uzbekistan, Pamiro-Altai Mts., Kulsai	TAAM 121360, holotype	LT594141		
Melanoleuca sp. Czechia	Czech Republic, Ivančice	BRNM 781065	LT594142	LT594178	LT594164
Melanoleuca sp. Korea	South Korea, Mongsanpo	BRNM 781059	LT594153	LT594180	LT594166

The European specimens previously identified as *M. angelesiana* (Vizzini et al. 2011) is unrelated to the syntype sequence of *M. angelesiana* (Antonín et al. 2021), which seems to be a strictly American species. Therefore, the European "*M. angelesiana*" lineage should be described as a new species. Unfortunately, five American specimens identified as *M. angelesiana* and available for this study did not resulted as *M. angelesiana sensu typi* either. Three of them from Colorado (DBG 28118, DGB 28732, and DGB 39834) belong to *M. acystidiata* lineage but are not fully identical with European *M. acystidiata*, so we name them *M. acystidiata* aff. USA. Other two *M.* aff. *angelesiana* specimens from Idaho (NY 1772313 and NY 1927565) grouped separately (*M. angelesiana* aff. USA in this paper).

Another previously unrecognized phylogenetic species is a lineage of four specimens from Italy (Emilia-Romagna, surroundings of Ravenna; specimens nos. BRNM 772193, BRNM 829059 – BRNM 829061), resulted as a sister species to *M. fontenlae* (= *M. pseudopaedida* sensu Vizzini et al. 2011) and also needs a formal description.

Taxonomy

Melanoleuca monticola Antonín, Ďuriška, Jančovičová, Para, Ševčíková & Tomšovský, **sp. nov.** (Figs. 3 and 6c)

Mycobank ***

= *M. angelesiana* A.H. Sm. s. Vizzini et al. (2011); ? *M. phaeopodia* s. Bres. (Bon 1991).

Diagnosis. It differs from closely related *M. brachyspora* by the absence of a caulohymenium, a distinctly clavate to bulbose stipe base and DNA sequences.

Holotype. Slovakia, Vysoké Tatry Mts., Podbanské, Kôprová dolina, on a disturbed soil with stones on the margin of a road, 25 Oct. 2012 leg. O. Ďuriška (SLO1556).

Etymology. Known only from montane and hilly areas.

Pileus 25–175 mm broad, plano-conical or convex with distinct, obtuse umbo and involute at margin, then applanate with distinct, broad, low umbo and inflexed or reflexed margin, pileipellis exceeding the lamellae margin, usually not striate, sometimes translucently striate, smooth or very slightly rugulose at centre, sometimes with small depressions, smooth, finger test 0, glabrous or pruinose, lustrous, ± uniformly silvery grey, pale brown-grey (Y70M50C50), grey-brown (6–7E3–5, Y90M80C80), or (dark) brown (6C–D5–6, Y90M70C60) with darker, brown (7E–F6, Y90M80C70), dark brown (Y99M90C80) to black-brown (7F5–6, S99Y40M40) centre. Lamellae rather close, L = c. 45–80, l = 1–4, emarginate and attached with tooth, sometimes furcate near stipe, ± horizontal to ventricose, white (S00Y00M00), whitish (between S00Y00M00 and S00Y10M00) to pale cream coloured (Y30M10C10), with beige or greyish tinge or without it, with concolorous, finely pubescent edge. Stipe 30–80(–155) × 3–17 mm, cylindrical, slightly broadened at apex, distinctly clavate (up to 20 mm) or bulbous (with bulb up to 30 mm), sometimes entirely clavate when young, or not broadened at base, rather distinctly longitudinally fibrillose, or longitudinally striate, sometimes twisted, white at apex, grey-brown (6C–E4–6) otherwise, entirely, especially at apex floccose, floccules white at apex, grey-brown towards base; with white basal tomentum. Context white (S00Y00M00), whitish (between S00Y00M00 and S00Y10M00), cream (Y40M20C20), sometimes to pale brown (Y50M40C30) in pileus, white (S00Y00M00), whitish (between S00Y00M00 and S00Y10M00), cream (Y40M20C20) to pale brown (Y50M40C30) in stipe, sometimes darker than apex in the stipe base, with fungoid smell or without any or with slight earthy smell and with mild (sometimes sweetish) taste.

Basidiospores 5.5–10 × 4.0–6.5(–7.0) µm, average = 7.15 × 5.10 µm, E = (1.0–)1.17–1.73, Q = 1.25–1.59, (broadly) ellipsoid, rarely globose, thin-walled, ornamentation verruculose with scattered cristulae, amyloid. Basidia 30–55 × 10–12 µm, 4-spored, clavate. Basidioles 15–50 × 5.0–13 µm, clavate, cylindrical. Cheilo- and pleurocystidia not seen. Marginal cells absent or 18–31 × 4.5–11 µm, clavate, cylindrical, irregular or irregular, thin-walled. Trama hyphae cylindrical to ellipsoid, thin-walled, non-dextrinoid, 3.0–15(–25) µm wide. Pileipellis an ixocutis, sometimes transient to trichoderm, composed of ± radially arranged, cylindrical, slightly gelatinized (especially at centre), smooth or rarely minutely

incrusted, thin-walled, 4.0–10 µm wide hyphae with vacuolar and incrusting grey-brown or brown pigmentation; terminal cells adpressed to often (sub)erect (especially towards centre), cylindrical or narrowly clavate, subfusoid, sublageniform, thin-walled, 4.0–8.0 µm wide. Stipitipellis a cutis of cylindrical, parallel, ± thin-walled, 3.0–7.0 µm wide hyphae. Caulocystidia single or in groups, (17–)20–80 × 4.0–10 µm, clavate, cylindrical, thin- to slightly thick-walled; caulohymenium absent. Clamp connections absent.

Ecology. On soil in a stream alluvium under *Picea*, *Salix*, *Petasites* and *Urtica*, on a margin of a montane spruce forest under *Picea*, *Betula* and *Rubus*, in mosses under *Rubus* and *Picea*. In woodlands (under *Picea abies*, *Fagus sylvatica*) along roads and paths in leaves, wood or herb debris, on sawdust or in grass and among herbs (e.g. *Arctium*, *Taraxacum*, *Mentha*, *Rumex*, *Oxalis*, *Petasites*, and *Trifolia.* Often on anthropogenous places.

Other specimens examined.

CZECH REPUBLIC: Brdy Mts., Nová Ves, c. 500 m SE of the village, leg. J. Kasíková (BRNM 781198). – Novohradské hory Mts., Lužnický vrch, 30 Sep. 2008 leg. M.T. Basso (Antonín 08.257, BRNM 829077). – Novohradské hory Mts., Pohoří na Šumavě, spruce stand close to Prameniště Pohořského potoka Nature Monument, 28 Sep. 2008 leg. M. Beran (Antonín 08.232, BRNM 829076). ITALY: Trentino Prov., Malga Pura of Pieve di Bono, 13 Sep. 2002 leg. E. Bizio, R. Para and R. Fontenla 020913-02 (ANC M0144). SLOVAKIA: Západné Tatry Mts., Podbanské, Tichá dolina, vicinity of the Tábor cottage, alluvium of the Tichý potok stream, alt. 1040–1120 m, 28 Aug. 2003 leg. O Jindřich (Antonín 03.38, BRNM 829073). – Západné Beskydy, Mútne, Mútňanská píla, 10 Oct. 2012 leg. O. Ďuriška (SLO1525). – Javorníky Mts., Vysoká nad Kysucou, 12 Oct. 2012 leg. S. Jančovičová (SLO1542). – Vysoké Tatry Mts., Podbanské, Tichá dolina, 25 Oct. 2012 leg. S. Jančovičová (SLO1553). – Ibid. (SLO1559). – Ibid. leg. O. Ďuriška (SLO1555). – Ibid. 26 Oct. 2012 leg. O. Ďuriška (SLO1562). – Západné Tatry Mts., Podbanské, Kamenistá dolina, 24 Oct. 2012 leg. S. Jančovičová (SLO1546). – Ibid. leg. O. Ďuriška (SLO1547). – Vysoké Tatry Mts., Podbanské, 26 Oct. 2012 leg. S. Jančovičová (SLO1563). – Ibid. 25 Oct. 2012 (SLO1549). – Ibid. 25 Oct. 2012 (SLO1550). – Ibid. 25 Oct. 2012 leg. O. Ďuriška (SLO1548). – Vysoké Tatry Mts., Podbanské, Kôprová dolina, 25 Oct. 2012 leg. O. Ďuriška (SLO1558). – Ibid. (SLO1556). – Nízke Tatry Mts., Jarabá, 3 Nov. 2012 leg. O. Ďuriška (SLO1570). – Čergov, Hertník, 15 Sep. 2010 leg. P. Kešeľák (SLO1588). – Veľká Fatra Mts., Liptovské Revúce, Zelená dolina, 30 Sep. 2013, leg. O. Ďuriška (SLO1622). – Veľká Fatra Mts., Vlkolínec, Vrchlúka, 10 Sep. 2013 leg. M. Tomšovský (SLO1597). – Horná Orava LPA, Trstená, Za Jelešňou protected area, alt. 620–640 m, 1 Oct. 2000 leg. D. Janda and V. Antonín (Antonín 00.149, BRNM 653033). SWITZERLAND: Graubünden, Rhäzüns, gem. Thusis, Lag Miert, alt. 1300–1400 m, 30 Sep. 2004 leg. V. Antonín 04.227 and 04.228 (BRNM 829074 and BRNM 829075).

Remarks. *Melanoleuca monticola* is characterized by rather small to large basidiomata, often with a relatively robust stipe; a grey, pale brown-grey, grey-brown or (dark) brown pileus with darker, brown, dark brown to black-brown centre; white, whitish to pale cream lamellae, sometimes with beige or greyish tinge; a distinctly clavate, entirely (especially at apex) floccose, grey-brown stipe; a whitish, sometimes up to

pale brown context in the stipe base; a very variable size and shape of basidiospores; by the absence of cheilo-, pleurocystidia and caulohymenium; and a pileipellis in the form of an ixocutis, sometimes transient to a trichoderm. A typical caulohymenium is absent, however, extremely rare caulocystidia (only 1–2 in one preparation) with a shape resembling the *brevipes*-type cystidia with a septum, 19–50 × 4–5 µm large, were found in several collections (SLO1553, SLO1547, SLO1556).

Phylogenetically, *M. monticola* is a sister species of *M. brachyspora*. *Melanoleuca monticola* was identified as *M. angelesiana* A.H. Sm. by Vizzini et al. (2011) or published probably as *M. phaeopodia* s. Bres. by Bon (1991). However, our phylogenetic studies showed clear differences between *M. monticola* and syntype of *M. angelesiana*, and the latter species represents a North-American taxon related to *M. acystidiata* (Antonín et al. 2021). We studied several specimens identified as *M. angelesiana* from the North-American herbaria DGB and NY, and they represent two different taxa than true *M. angelesiana* (*M. acystidiata* aff. USA and *M. angelesiana* aff. USA in this paper).

In comparison to other European species having white context in the stipe base and lacking cheilo- and pleurocystidia, *M. brachyspora* Harmaja differs by the presence of a caulohymenium and less distinctly clavate stipe base, *M. paedida* has an ochraceous fawn to pale or dark grey-brown pileus, a shorter stipe, with barely pruinose surface at the apex only and smaller basidiospores (6.5–8.5 × 3.9–5.5 µm, average 7.3 × 5.1 µm) and *M. acystidiata* has an only slightly longitudinally fibrillose, more or less cylindrical stipe, sometimes with only indistinct whitish floccules and slightly broader basidiospores, (6.7–)7.2–10(–11) × 5–7.2(–7.5) µm, average 8.2 × 6.1 µm (Antonín et al. 2021).

Melanoleuca graminicola **(Velen.) Kühner & Maire** (Figs. 4a–d and 6b)

Tricholoma graminicolum Velen., České Houby: 244, 1920 (non *Tricholoma graminicola* Velen., Novitates mycologicae novissimae: 62, 1939). – *Melanoleuca graminicola* (Velen.) Kühner & Maire, Bulletin de la Société Mycologique de France 50: 18, 1934.

Type material. Czech Republic, Mnichovice, the way in the direction to Struhařov, grassy, sunny country lane, July 1926, leg. J. Velenovský (PRC, bottle 59a – marked as isotype, bottle 59b – marked as lectotype).

Epitype. Czech Republic, Proseč, Toulovcovy maštale Nature Reserve, on soil, roadside, under *Picea abies*, *Abies alba* and *Ulmus*, 19 Oct. 2013 leg. V. Antonín and H. Ševčíková (Antonín 13.385, BRNM 829064).

Pileus 20–74 mm broad, convex-conical, low conical to applanate to uplifted, with rather distinct, obtuse umbo at centre and depressed around it, involute, later inflexed at margin, smooth, glabrous or finely rugulose-granulose (lens), sometimes translucently striate, finger test 0, light brown (Y50M40C30), brown to dark-brown (Y80M60C50; Y80M80C70, 6D–E5–6, 6D4, 7D4), usually darker (6F5-6, Y99M80C80) at centre, pallescent to ochraceous beige (5B3). Lamellae moderately close, L = c. 50–60, l = 3–4 (irregular), emarginate and attached to decurrent with tooth, rather narrow, white (S00Y10M00) to whitish (S00Y10M00) or pale cream, sometimes with greyish tinge, with concolorous, uneven, finely pubescent

edge. Stipe 30–90 × 2–9 mm, cylindrical, slightly broadened at apex, clavate to with small bulb (up to 14 mm) at base, longitudinally fibrillose, floccose-pubescent at apex, pale brown (Y60M40C30), orangish brown, brown, pale brown-grey to brown (6C–D5, Y70M50C50, Y80M70C60), then dark (greyish) brown (7E3–4); basal tomentum whitish, rich. Context in pileus whitish, pale brown (Y60M40C40) to grey-brown (Y80M70C70), sometimes with rusty tinge (Y90M80C50), watery brown-grey when moist, in stipe apex whitish to pale brownish, grey-brown (Y80M80C80) to brown (Y80M80C70), sometimes with rusty tinge (Y90M70C50), in the stipe base brownish, light to dark brown (Y90M80C60, Y90M90C90) to black-brown (S99Y99M20), sometimes rusty brown, rarely dirty whitish, without any distinct smell or slightly earthy, and with mild taste.

Basidiospores (5.5–)7.0–8.5(9.5) × 4.0–6.5 µm, average = 7.39 × 5.11 µm, E = (1.09–)1.20–1.78, Q = 1.33–1.58, ellipsoid, less frequently fusoid-ellipsoid, verruculose, warts isolated, rarely connected, up to 1.0 µm high, amyloid. Basidia 18–41 × 7.0–12 µm, 4-, rarely 2- or 1-spored, clavate. Basidioles 13–38 × 5.0–12 µm, clavate, rarely subcylindrical or subvesiculose or resembling cheilocystidia. Cheilocystidia scattered, urticoid, more or less of the *exscissa*-type, 18–50 × 5.0–11.0 × 3.0–5.0 µm, ± lageniform, often irregular, apex subulate, obtuse, thin-walled. Marginal cells 12–45 × 5.0–11 µm, clavate, (sub)cylindrical, (sub)utriform, sometimes irregular, thin-walled. Pleurocystidia absent. Trama hyphae cylindrical or subinflated, thin-walled, 3.0–15(–20) µm wide. Pileipellis an ixocutis transient to ixotrichoderm (centre) composed of cylindrical, ± thin-walled, non-dextrinoid, up to 10.0 µm wide hyphae; terminal cells adpressed to erect, (sub)clavate or cylindrical, rarely branched, obtuse, up to 60 × 7.0 µm; yellowish grey in KOH. Stipitipellis a cutis of cylindrical, parallel, ± slightly thick-walled, smooth or minutely incrusted, non-dextrinoid, 2.0–7.0 µm wide hyphae. Caulohymenium of (1) caulocystidia, 22–70 × 5.0–10 µm, lageniform, cylindrical, clavate, subfusoid, regular or irregular, thin- or slightly thick-walled, and (2) rare urticoid caulocystidia, 45 × 12 × 3.2 µm. Clamp connections absent.

Ecology. On soil in an alluvium of a stream, in *Urtica*, *Cirsium* and *Mentha* growth, among wood scrap, leaves debris, and in grass and moss among different herbs (*Carduus* sp., *Petasites* sp., *Traxacum* sp., *Urtica dioica*), on a margin of a wet spruce forest, under trees and shrubs (*Abies, Alnus*, *Corylus*, *Picea, Pinus*, *Salix*, and *Ulmus*) or on forest paths and roads margins.

Other specimens examined.

Austria: Gesäuse, Johnsbach, Schafgraben, 25. Aug. 2010 leg. H. Forstinger (Antonín 10.233, BRNM 829063). ITALY: Trentino Prov., Albarè of Pergine Valsugana, alt. 500 m, 4 Oct. 2002 leg. C. Piuri, R. Fontenla & R. Para 021004-04 (ANC M0145). SLOVAKIA: Strážovské vrchy Mts., Pružina-Priedhorie, Strážov National Nature Reserve, alt. 600–700 m, 7 Oct. 2005 leg. M. Vašutová (Antonín 05.210, BRNM 829062). – Horná Orava Landscape Protected Area, Trstená, Za Jelešňou Nature Reserve, 1. Oct. 2000 leg. V. Antonín 00.150 (BRNM 653028). – Strážovské a Súľovské vrchy, Podskalie, 6. Oct. 2005 leg. L. Hagara (BRACR15528, (BRACR15529). – Západné Beskydy, Mútne, Mútňanská píla, 10. Oct. 2012 leg. O. Ďuriška (SLO1528, SLO1531, SLO1527, SLO1525). – Ibid., leg. S. Jančovičová (SLO1532, SLO1526). – Západné Beskydy, Oravská Polhora, Slaná voda, 9. Oct. 2012 leg. S. Adamčík (SLO1518, SLO1522). –

Ibid., leg. O. Ďuriška (SLO1523). — Strážovské vrchy, Pevník časť Dolina, 11. Oct. 2013 leg. M. Caboň (SLO1637). — Ibid., leg. S. Jančovičová (SLO1634, SLO1635). — Veľká Fatra, Liptovské Revúce, Suchá dolina, 29. Sep. 2013 leg. O. Ďuriška (SLO1619, SLO1615). — Veľká Fatra, Liptovské revúce, Zelená dolina, 30. Sep. 2013 leg. O. Ďuriška (SLO1626, SLO1625, SLO1623). SPAIN: Teruel-Bronchales, 18. Oct. 2014 leg. E. Suarez (herb. E. Suarez H.H.T.S.G.B. 649C)

Remarks. *Melanoleuca graminicola* is characterized by rather small basidiomata with a light brown, brown to dark-brown and usually darker pileus at centre, pallescent to ochraceous beige; white, whitish to pale cream coloured lamellae; a pale brown, orangish brown, brown, pale brown-grey to brown, then dark (greyish) brown stipe, clavate or with small bulb, floccose-pubescent at apex; brownish, light to dark brown to black-brown, sometimes rusty brown, rarely dirty whitish context in the stipe base; moderately large basidiospores; scattered, urticoid cheilocystidia; and the present caulohymenium. Urticoid caulocystidia may rarely absent. One specimen (BRNM 653028) has two sizes of basidiospores: 10—12 × 6.0—8.0 µm (originating from 2-(1-)spored basidia), and 7.0—9.5 × 4.7—6.5 µm (originating from 4-spored basidia); the size of the second ones fit well to the size limit of this species.

This species is sometimes erroneously considered a synonymum of *M. angelesiana* A.H. Sm. by American authors (e.g. Gillman & Miller 1977).

Phylogenetically close species with brown stipe base context, *M. romanensis* differs from *M. graminicola* by the larger basidiospores (7.5–10 × 5.0–7.0 µm, average = 8.6 × 5.7 µm) and cheilocystidia mostly of the *brevipes*-type, smaller marginal cells [12–30 × (3.5—)6.0–11 µm]; *M. fontenlae* Para, Antonín, Ďuriška, Ševčíková & Tomšovský by the larger ellipsoid basidiospores [(6.0—)6.5–10 × (4.0—)4.8–6.0(—6.5) µm, average = 8.0 × 5.6 µm] broadly) with mostly irregularly shaped and sometimes up to 0.75 µm high warts and sometimes with rare ridges and cheilocystidia of both the brevipes- and exscissa-type (Antonín et al. 2021)d *microcephala* (P. Karst.) Singer by a pileus (greyish) brown at centre and beige-grey or greyish brown otherwise and the absence of cheilocystidia and urticoid caulocystidia (Antonín et al. 2021).

Melanoleuca rufipes **Bon**, Documents Mycologiques 8(29): 34, 1978.

Holotype. France, Gard, Tarascon (Pichegu), Oct. 1977 leg. M. Bon (LIP, Bon 771029).

Macroscopic description. Bon (1978).

Holotype revision. Basidiospores (5.8—)6.5—7.5(—8.0) × 4.5–6.0 µm, average = 7.0 × 5.2 µm, E = 1.23–1.44, Q = 1.28, broadly ellipsoid, subovoid, subglobose, verruculose, warts variable in shape and size, irregular. Basidia 23–38 × 11–12 µm, 4-spored, clavate. Basidioles up to 33 × 6.0–13 µm, clavate. Cheilocystidia (only a few seen, mostly collapsed) urticoid, mostly of the *brevipes*-type, rarely of the *exscissa*-type, 20–30 × 7.0–10.5 µm, basal part clavate or fusoid, sometimes irregular, apical part cylindrical to subulate, thin-walled, without apical crystals. Marginal cells 13–18 × 6.0–8.5 µm, clavate, subcylindrical, sometimes irregular, thin-walled. Pleurocystidia not seen. Trama hyphae ± cylindrical, thin-walled, non-dextrinoid, up to 12(—15) µm wide. Pileipellis a cutis composed of ± cylindrical, interwoven,

thin-walled, non-dextrinoid, 3.0–9.0 µm wide hyphae; terminal cells adpressed, rarely suberect, cylindrical, narrowly clavate, obtuse, thin-walled; vacuolar pigment brown. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth, up to 6.0 µm wide hyphae. Caulocystidia 14–30 × 8.0–12 µm, clavate or subfusoid, less frequently subcylindrical, ± irregular, thin-walled. Clamp connections absent.

Ecology. On soil under *Cupressus arizonica*.

Remarks. According to the original macroscopic description (Bon 1978a) *rufipes* differs from newly proposed *M. romanensis* (see below) by the presence of purpurascent or red tinges at stipe base and rusty brown stipe base context. Microscopically (holotype) it has smaller, (5.8–)6.5–7.5(–8.0) × 4.6–6.0 µm (average 7.0 × 5.2 µm), and distinctly broader ellipsoid (Q = 1.28) basidiospores, shorter, 20–30 × 7.0–10.5 µm large cheilocystidia and the absence of a caulohymenium. Therefore, the latter taxon is described as a new species below.

Microscopically, the closest species seems to be *M. castaneofusca* with (6.0–)6.5–8.0 × 4.0–6.0 µm (average 7.2 × 4.9 µm, Q = 1.47) large basidiospores. However, it differs by a pileus dark grey-brown to brown or pale brown with a darker brown centre, a stipe brownish to grey-brown or dark brown and distinctly floccose to floccose-tomentose at apex, a paler coloured context in the stipe base, larger cheilocystidia (18–55 × 5.0–11 µm) and the presence of a caulohymenium (Antonín et al. 2021).

For a final confirmation of the taxonomic position of this species, it is necessary to obtain recent collections of the species, preferably those from the type locality or its vicinity in France, to provide detailed morphological and molecular analyses.

Melanoleuca romanensis Antonín, Ďuriška, Jančovičová, Para, Ševčíková & Tomšovský, **sp. nov.**
(Figs. 4e–f and 6d)

MycoBank MB ******

Diagnosis. Melanoleuca fontenlae differs by a smaller, 9–39 mm broad, whitish grey, grey-brown or dark brown pileus pallescent up to ochraceous brown to brown, an ochraceous yellowish to brownish stipe at centre and larger cheilocystidia (19–65 × 3.0–10 µm).

Holotype. Ravenna, Lido di Dante, on sandy dunes under *Pinus* and *Quercus*, 9 Nov. 2000 leg. V. Antonín 00.239 and A. Hausknecht (BRNM 772193).

Etymology. Named after eastern part of the Emilia-Romagna region, Italy, the area of distribution of the species.

Pileus 15–55 mm broad, broadly conical with distinct obtuse central umbo and inflexed to shortly involute margin, then ± applanate with small sometimes up to indistinct central umbo and depressed around it and with inflexed margin, margin sometimes shortly costate, hygrophanous, not translucently striate or shortly translucently striate at margin only, smooth, glabrous, but sometimes apparently finely

tomentose at centre, finger test 0, light brown to dark brown (6D4 to 7E5, 6E–F6) at centre and paler, brownish orange, brown (6C3 to 6D–E4, 6–7E3–6) towards margin, sometimes ivory greyish, whitish or blackish. Lamellae moderately close, L = c. 40–50, l = 2–4, emarginate and attached with tooth, mostly sinuate, sometimes furcate near stipe, white or yellow cream coloured, with slightly beige reflex, with concolorous, finely pubescent edge. Stipe 25–60 × 2.5–7 mm, cylindrical or slowly broadened towards base, straight or curved, slightly broadened at apex, subcylindrical to clavate-bulbose (up to 12 mm) at base, finely pubescent to flocculose at apex, longitudinally (sometimes silvery) (distinctly) fibrillose otherwise, sometimes twisted, brownish orange (6C4) at apex, darker, brown to dark brown (6D–E4–5, ±7D4) or greyish brown with fleshy tinge towards base; basal tomentum white. Context whitish in pileus, brownish under pileipellis, fibrillose in stipe, orangish brown, dark brown (7D7) in base, with indistinct to earthy smell and mild but astringent taste.

Basidiospores 7.5–10 × 5.0–7.0 µm, average = 8.6 × 5.7 µm, E = 1.29–1.8, Q = 1.41–1.59, (broadly) ellipsoid, fusoid-ellipsoid, verruculose, warts variable in size, ± regular. Basidia 30–39 × (8.0–)10–12 µm, 4-spored, clavate. Basidioles 15–40 × 5.0–13 µm, clavate, subcylindrical, subfusoid. Cheilocystidia urticoid, mostly of the *brevipes*-type, 27–48 × 6.0–9.0 µm, basal part fusoid, subcylindrical, clavate, sometimes irregular, apical part 2.5–3.0 µm wide, cylindrical to subulate, thin-walled, muricate or not. Marginal cells 12–30 × (3.5–)6.0–11 µm, clavate, fusoid, (sub)cylindrical, vesiculose, often irregular, thin-walled. Pleurocystidia not seen. Trama hyphae cylindrical to (sub)inflated, thin-walled, non-dextrinoid, up to 20 µm wide. Pileipellis an (ixo)cutis (margin) to ixotrichoderm (centre) composed of cylindrical, ± thin-walled, non-dextrinoid, up to 10 µm wide hyphae; terminal cells adpressed to suberect, 17–58 × (2.0–)6.0–12 µm, cylindrical, narrowly clavate, (sub)fusoid, obtuse, thin-walled. Stipitipellis a cutis of cylindrical, parallel, ± thin-walled, smooth, up to 7.0 µm wide hyphae. Caulohymenium of (1) 17–43 × 5.0–9.0(–11) µm, cylindrical, clavate or subfusoid, sometimes irregular, thin-walled cells, and (2) urticoid to hair-like cystidia, (25–)33–40 × 2.5–5.0(–6.0) µm, fusoid, narrowly lageniform, rostrate, subulate, thin-walled usually without apical crystals. Clamp connections absent.

Ecology. On sandy soil in grass under *Pinus pinaster*, *P. pinea*, *Quercus ilex*, *Q. pubescens* and *Q. robur*.

Other specimens examined.

ITALY: Ravenna, Pineta Ramazzotti and dunes di Lido di Dante, alt − 14 m, 5 Nov. 2007 leg. V. Antonín 07.410 (BRNM 829059). – Ravenna, Pineta di Classe, part Pinarella-Bosconi, alt. − 10 m, 6. Nov. 2007 leg. V. Antonín 07.424 (BRNM 829060). – Ravenna, Pineta di Classe, Anello di Dante, alt. − 12 m, 9. Nov. 2007 leg. V. Antonín 07.444 (BRNM 829061). – Lazio Prov., Sabaudia, in a wood of *Quercus ilex* and *Pinus* on sandy soil, behind the dunes, alt. 7 m, 17 Nov. 2006, leg. R. Fontenla & R. Para (ANC M0199). – Ravenna Prov., mouth of the river Bevano of Ravenna, in a mixed forest with *Pinus pinaster*, *Quercus pubescens* and *Q. ilex*, on sandy soil, alt. 5 m, 6 Jan. 2007, leg. G. Consiglio, G. Perdisa, R. Fontenla & R. Para (ANC M0200)

Remarks. *Melanoleuca romanensis* is characterized by rather small basidiomata with a light brown to dark brown pileus at centre and paler, brownish orange, brown towards margin; yellow cream lamellae; a

subcylindrical to clavate-bulbose stipe brownish orange at apex, darker and brown to dark brown towards base; an orangish brown or dark brown context in the stipe base; 7.5–10 × 5.0–7.0 µm basidiospores; present urticoid cheilocystidia; absent pleurocystidia; and present caulohymenium.

Phylogenetically and ecologically close *M. fontenlae* differs by a smaller, 9–39 mm broad, whitish grey, grey-brown or dark brown pileus pallescent up to ochraceous brown to brown, an ochraceous yellowish to brownish stipe at centre and larger cheilocystidia (19–65 × 3.0–10 µm); Antonín et al. 2021. *M. graminicola* (Velen.) Kühner & Maire differs by a rather long and slender stipe (50–90 × 3–6 mm) and smaller basidiospores (7.0–8.5(–9.5) × 4.5–5.5 µm, average = 7.9 × 5.0 µm).

Melanoleuca juliannae var. *decolorans* has a smaller, 25–35 mm broad, entirely dark brown pileus then pallescent to (grey-)brown from margin, a smaller, 28–45 × 3–6 mm stipe, cylindrical to slightly clavate-bulbose (up to 7 mm) at base, dark grey-brown with brown apex (Antonín et al. 2014). *Melanoleuca luteolosperma* has a uniformly pale ochraceous grey to grey, greyish brown or brown pileus with whitish outermost margin and pale ochraceous yellow or pale grey-brown stipe and slightly smaller basidiospores [(6.0–)7.0–9.0 × (4.0–)4.2–5.5(–6.5) µm; average 7.7 × 5.1 µm]; Antonín et al. 2021. *Melanoleuca humilis* differs by a grey-brown pileus, greyish or beige lamellae, a stipe dark watery brown when moist and greyish when dried-out and it mostly grows on ruderalised or anthropogenous habitats (Antonín et al. 2015).

Melanoleuca brachyspora **Harmaja**, Karstenia 25: 44, 1985. (Figs. 5a–d and 6a)

= *Melanoleuca brevispora* Harmaja, Karstenia 18: 30, 1979, non *M. brevispora* Singer, Sydowia 8: 115, 1954.

Holotype. Finland, Varsinais-Suomi (V/Ab). Karkkila (Pyhäjärvi), Haavisto, fen 500 m E of the small lake Iitalampi, 25 Sep. 1969 leg. H. Harmaja (H 6003414!).

Pileus 30–85 mm broad, convex or plano-convex, with applanate centre with almost indistinct or rather distinct broad obtuse umbo, margin reflexed and involute, not translucently striate or only slightly striate at margin, smooth, glabrous, finger test 0, entirely brownish grey (6D3, Y50M40C40) or brown to dark brown (6E7–8, 7E–F6–7, Y70M50C40) and sometimes light brown (6–7D5, Y50M30C2) at margin or sometimes with the whitish outermost margin (in places where pileipellis reach beyond lamellae). Lamellae moderately close, L = c. 40–60, l = 2–7 (irregular), emarginate and attached to shortly decurrent with tooth, ± horizontal, whitish (between S00Y00M00 a S00Y10M00), dirty cream or yellowish cream (4A3, S10Y40M10) coloured, sometimes with greyish tinge, with concolorous, uneven, finely pubescent edge. Stipe 45–100 × 4.5–10 mm, cylindrical, slightly broadened at apex, subcylindrical to clavate (7–15 mm) at base, longitudinally fibrillose, finely floccose or pubescent at apex, whitish, later pale grey-brown or light brown (6–7D4, 7D3–4, 7D–E7, 6C4, Y50M30C20; Y70M50C40); basal tomentum white. Context watery whitish, fibrillose in stipe, white in stipe, without any darker base, under pileipellis brown, grey-brown in stipe cortex, with fungoid smell and mild, after short time adstringent taste.

Basidiospores (5.0–)6.0–9.0(–9.5) × (3.8–)4.5–7.0 µm, average = 7.32 × 5.28 µm, E = (1.09–)1.17–1.70, Q = 1.25–1.47, broadly ellipsoid, subovoid, subamygdaloid, irregularly verruculose (warts up to 0.75 µm high), with rare connections, warts up to 0.5 × 0.5 µm, amyloid. Basidia 16–42 × 9.0–12 µm, 4-spored, clavate or subfusoid. Basidioles 14–41 × 4.0–12 µm, clavate to cylindrical. Cheilocystidia and pleurocystidia not seen; cystidioid hairs rarely present. Marginal cells 15–36 × 3.5–12 µm, cylindrical, clavate, fusoid, mostly irregular to subcoralloid, thin-walled, sometimes scattered. Trama hyphae cylindrical to subinflated, thin-walled, 3.0–15 µm wide. Pileipellis an (ixo)cutis, sometimes transient to (sub)ixotrichoderm at centre, composed of radially arranged, cylindrical or subfusoid, smooth or minutely incrusted, 4.0–10 µm wide hyphae; terminal cells adpressed to (sub)erect, narrowly clavate, cylindrical, subfusoid, obtuse, thin-walled, up to 70 × 4.0–10 µm wide. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, 3.0–7.0 µm wide hyphae. Caulohymenium of (1) cystidia 15–60(–95) × 2.0–12 µm, in groups, (long) cylindrical, (narrowly) clavate, (narrowly) fusoid, thin-walled, and (2) basidia, 31–38 × 8.0–11 µm, 4-spored, clavate, sometimes seem absent. Clamp connections absent.

Ecology. On soil in grass and mosses under *Picea abies* and *Salix* sp., in mossy grass under *Pinus* on serpentinite, and a tree clearing, along the forest path, and a fire place in a spruce forest.

Other specimens examined.

CZECH REPUBLIC: České Švýcarsko National Park, Doubice, Divoká rokle valley, 13 Oct. 2010 leg. V. Antonín 10.384 and S. Komínková (BRNM 772200). – Ibid., leg. V. Antonín 10.391 (BRNM 829072). – Český Šternberk, Vrábov, 4 Nov. 2011 leg. J. Borovička (BRNM 829069). – Bernartice – Borovsko, Hadce u Želivky National Nature Monument, 26 Oct. 2012 leg. J. Borovička (BRNM 829067). – Ibid., 26. Nov. 2014 leg. J. Borovička (BRNM 829068). – Bernartice, 11 Nov. 2021 leg. J. Borovička (PRM 958041). – Novohradské hory Mts., Malonty, Hodonický potok, 29 Sep. 2003 leg. V. Antonín 03.79 (BRNM 829070). DENMARK: Ronde, 19. Oct. 2012 leg. R. Ejrnaes (SLO1691). SLOVAKIA: Veporské vrchy Mts., Hriňová, Biele Vody, alt. 900–1000 m, 26 Sep. 2009 leg. J. Holec (Antonín 09.283, BRNM 829071). – Vysoké Tatry Mts., Podbanské, 25 Oct. 2012 leg. S. Jančovičová (SLO1552). – Ibid., 26 Oct. 2012 leg. O. Ďuriška (SLO1565, SLO1564). – Vysoké Tatry Mts., Tichá dolina, 25. Oct. 2012 leg. O. Ďuriška (SLO1554). – Poľana, Spálený vrch, 16. Sep. 1995 leg. S. Glejdura (M363, SLO1685).

Remarks. *Melanoleuca brachyspora* has a brownish grey, brown to dark brown pileus sometimes light brown or whitish at margin; dirty cream or yellowish cream lamellae; a pale grey-brown or light brown stipe; a white context in the stipe base; rather broad basidiospores; absent cheilocystidia; and present caulohymenium composed of simple cystidia and basidia. The holotype specimen (H 6003414) differs by the distinctly smaller basidiospores [(4.8–)5.5–7.0(–7.5) × (3.8–)4.5–6.6 µm, average 6.2 × 4.9 µm] which are more distinctly subglobose to broadly ellipsoid (E = 1.10–1.40, Q = 1.26).

The only one cheilocystidium of the *brevipes*-type, 29 × 6 µm, was observed in the specimen SLO1565 (Slovakia, Podbanské, M229) as a result of the observation of several preparations. Likewise, caulocystidia of the *brevipes*-type, 15–77 × 3.0–20 µm, with or without septum, were observed in specimens SLO1552, SLO1564 and SLO1554 (all from Slovakia, Podbanské), always the only one-two in

each specimens. Because these cystidia were very rare and always unusually twisted, and may represent some aberrant cells.

Related *M. stridula* differs by a brownish to brown context in the stipe base. Morphologically very close *M. monticola* differs by an always distinctly clavate or bulbose stipe base and the absence of the caulohymenium.

Compared to other species without cystidia, *M. acystidiata* differs by slightly larger basidiospores [(6.7–)7.2–10(–11) × 5–7.2(–7.5) µm, average 8.2 × 6.1 µm] (Antonín et al. 2021); M. *microcephala* differs by a differently coloured, centrally (greyish) brown or beige-grey or greyish, otherwise uniformly greyish brown pileus, a stipe usually longer than the pileus width, 22–105 × 2–5.5 mm, a pale to dark (reddish) brown to black-brown context in the stipe base and slightly larger basidiospores [7.0–9.5(–10) × 5.0–7.0 µm, average 8.3 × 5.8 µm] (Antonín et al. 2021).

Melanoleuca stridula **(Fr.) Singer**, Cavanillesia 7: 129 (1935). (Figs. 5e and 6c)

Agaricus stridulus Fr., Epicrisis Systematis Mycologici: 85, 1836. – *Collybia stridula* (Fr.) Quél., Mémoires de la Société d'émulation de Montbéliard II, 5: 237, 1872. – *Gyrophila stridula* (Fr.) Quél., Enchiridion Fungorum: 28, 1886. – *Tricholoma stridulum* (Fr.) Sartory & Maire, Synopsis du genre *Tricholoma*: 26, 1918. – *Oudemansiella stridula* (Fr.) M.M. Moser, Zeitschrift für Pilzkunde 19: 9, 1955 (comb. inval.).

Neotype. Italia, Posatora (AN), growing on the ground, among pine needles and the grass, under *Pinus halepensis*, 200 m a.s.l., leg. M. Gottardi, det. R. Fontenla, M. Gottardi, R. Para (ANC M0007, Fontenla et al. 2003).

Pileus 18–50 mm broad, broadly conical, almost applanate to applanate, depressed or slightly depressed at centre with low and broad umbo, surface of pileus sometimes with small depressions, straight, inflexed to involute at margin, finger test 0, glabrous, apparently slightly pruinose at margin, finely granulose-rugulose (lens), uniformly rather dark (grey-)brown or dark brown (6E4–5, 6–7E5–7, 7E–F6, Y90M70C60, S30Y40M20), sometimes slightly darker (7F6) at centre. Lamellae rather close, L = c. 35–50, l = 3–5, emarginate and attached to decurrent with tooth, sinuate, rather narrow (up to 6 mm), cream coloured with beige reflex, edge concolorous, pubescent, sometimes uneven. Stipe 40–95 × 2.5–6 mm, cylindrical, slightly broadened at apex, cylindrical, but mostly clavate to subbulbose (up to 11 mm) at base, finely floccose-pubescent at apex, entirely (distinctly) longitudinally striate, dirty whitish, brownish or grey-brownish (Y70M50C40) at apex, grey-brown to dark brown (6D5, 6E3–4, 7–8E3–4, Y90M80C80) towards base; basal tomentum white. Context whitish in pileus, fibrillose, whitish to grey-brown in stipe, brownish to dark brown or black-brown (Y90M80C80, Y90M90C90) in stipe base, with indistinct or slightly fungoid smell and mild, sometimes slightly adstringent taste.

Basidiospores 6.0–8.5(–9.0) × 4.0–6.0 µm, average = 7.3 × 5.2 µm, E = 1.16–1.80, Q = 1.32–1.59, (broadly) ellipsoid, subovoid, verruculose, warts up to 0.75 µm high irregular in size and shape, amyloid. Basidia (15–)29–40 × 7–12 µm, 4-spored, clavate. Basidioles 12–35 × 4.0–12 µm, clavate, cylindrical.

Cheilo- and pleurocystidia not seen; rarely rostrate marginal cells slightly similar to urticoid cheilocystidia present. Marginal cells 11–40(–45) × 4.0–13 µm, clavate, (sub)cylindrical, subfusoid, mostly irregular, thin-walled. Trama hyphae cylindrical to subinflated, thin-walled, hyaline, non-dextrinoid, up to 12(–20) µm wide. Pileipellis an ixocutis to subixotrichoderm (centre) composed of cylindrical, ± thin-walled, radially arranged, smooth or incrusted, non-dextrinoid, up to 10 µm wide hyphae; terminal cells adpressed to erect, up to 50 × 12 µm, clavate, subfusoid, cylindrical, thin-walled; pigmentation grey in KOH. Stipitipellis a cutis of cylindrical, parallel, ± thin-walled, up to 8.0 µm wide hyphae. Caulocystidia 20–80 × 3.0–8.0 µm, sometimes in form of urticoid cystidia of the *exscissa*-type, cylindrical, subulate, obtuse, thin-walled, sometimes with crystals (found in one collection), 15–38 × 5.0–8.0 µm, clavate, cylindrical, subfusoid, thin-walled (in three collections). Clamp connections absent.

Ecology. On soil in grass (*Molinia*), along the roads (under *Picea abies* and *Pinus sylvestris*) near a stream (*Picea*, *Sorbus aucuparia*), and in mosses on spruce wood scrap, and under *Pinus* on sandy soil, and under *Larix decidua* in a montane forest.

Other specimens examined.

AUSTRIA: Tyrol, Ehrwald, 4 Sep. 2011 leg. H. Huijser (Antonín 11.121, BRNM 825717). FRANCE: Hautes Alpes Dept., Arvieux, Lac de Roue, 1850 m alt., 15 Aug. 2019 leg. F.-X. Boutard (BRNM 829065). ITALY: Ravenna Prov., Parco Regionale del Delta del Po, Bedalassona, − 3 m alt., 4 Nov. 2007 leg. A. Verbeken and V. Antonín 07.406 (BRNM 761903). SLOVAKIA: Strážovské vrchy LPA, Suľovské vrchy hills, Podskalie, a stream valley SE of Podskalský Roháč Mt., alt. 480–550 m, 6 Oct. 2005 leg. V. Antonín 05.204 and M. Vašutová (BRNM 825716). – Belianské tatry Mts., Tatranská Kotlina, Dolina siedmich prameňov valley, 1150–1300 m alt., 6 Sep. 2001 leg V. Antonín 01.241 (BRNM 761902). – Považský Inovec, Bojná, 1. Nov. 2007 leg. L. Hagara (BRA CR 15526 and BRNM 772202). – Liptovská kotlina, Hybe, 21. Oct. 2012 leg. V. Kautman (SLO1543). – Vysoké Tatry, Kôprová dolina, 25. Oct. 2012 leg. S. Jančovičová (SLO1557). – Čergov, Sedlice, 18. Oct. 2008 leg. P. Kešeľák (SLO1586).

Remarks. *Melanoleuca stridula* has small to moderately large basidiomata; a rather dark (grey-)brown or dark brown pileus, sometimes slightly darker at centre; an entirely (distinctly) longitudinally striate, grey-brown to dark brown stipe; a brownish to dark brown or black-brown context in the stipe base; absent cheilocystidia; and absent caulohymenium. The presence of several cheilocystidia of the *brevipes*-type, 17–35 × 6–10 µm, were observed in the collection BRA CR 15526.

The detailed historical overview of *M. stridula*, including the neotype proposal, was published by Fontenla et al. (2003). Lange (1933) described *Tricholoma stridulum* var. *pallidipes* J.E. Lange. This taxon was considered a form (Favre 1948), later a variety of *M. stridula* (Bon 1978), and, finally a separate species *M. pallidipes* (J.E. Lange) Bon (comb. inval., Bon 1991). However, Lange (1935) drawn this species with smooth spores. Therefore, it does not belong to the genus *Melanoleuca*.

Related *M. brachyspora* differs by a white context in the stipe base. *M. monticola* differs by more robust basidiomata, slightly larger basidiospores and the constant absence of the caulohymenium.

Compared to other species without cystidia, *M. acystidiata* differs by larger basidiospores [(6.7–)7.2–10(–11) × 5–7.2(–7.5) µm, average 8.2 × 6.1 µm] (Antonín et al. 2021); M. *microcephala* differs by a differently coloured, greyish brown, beige-grey or greyish pileus, slightly larger basidiospores [7.0–9.5(–10) × 5.0–7.0 µm, average 8.3 × 5.8 µm] (Antonín et al. 2021).

Doubtful Taxa

Melanoleuca electropoda Maire & Malençon, in Malençon & Bertault, Champignon Supérieurs du Maroc 33: 77, 1975.

≡ *Melanoleuca paedida* f. *electropoda* (Maire & Malençon) Fontenla, Para & Vizzini, Mycotaxon 118: 376, 2011.

Holotype. Morocco, Bou-Jirirh, 4. Nov. 1943, herb. G. Malençon 1425, MPU.

Type revision. Basidiospores 6–7.4 × 4.8–6 µm, average 6.70 × 5.48 µm, E = 1.02–1.50, Q = 1.23, small, subglobose to shortly ellipsoid, with large, isolated, round warts, amyloid. Cheilocystidia rare, typically urticoid, with abundant apical crystals. Pleurocystidia not observed. Stipitipellis not observed. Pileipellis a cutis of interwoven hyphae.

Remarks. Vizzini et al. (2011): Subclade A4.2 (1.0 BPP, 99% MLB) encompasses two specimens of *M. paedida*, *M.* sp. 2 (specimen ANC M0188; JN616477), and *M. electropoda* (specimen ANC M0187; JN616430). The two *M. paedida* collections are consistent with the protologue and the observations by Fontenla et al. (2003). *Melanoleuca* sp. 2 is an acystidiate form of *M. paedida*. *Melanoleuca electropoda* was reported by Bon (1991) as a macrocystidiate species (subg. *Melanoleuca*, sect. *Oreineae*). After observation of typical urticoid cheilocystidia in the type collection of *M. electropoda*, Fontenla et al. (2003) considered *M. rufipes* Bon a later synonym of this species (see comments about *M. rufipes* above). Based on DNA sequence data, both sequences JN616430 and JN616477 of *M.* sp. 2 and *M. electropoda* group with *M. paedida*. The differences in several nucleotides among the sequences are crowded at the beginning or the ends of the sequences and very probably are caused by sequencing errors undetected before the sequence analyses. Both species are close also morphologically; *M. paedida* differs only slightly by the pileus colour and always white lamellae. Therefore *M. electropoda* can represent only a form of *M. paedida* as proposed by Vizzini et al. (2011).

Melanoleuca striimarginata Métrod ex Bon, Documents Mycologiques 20 (79): 59, 1990.

≡ *Melanoleuca striimarginata* Métrod, Revue de Mycologie 7: 94, 1942, nom inval.

Holotype. France, Champagnole, in small groups among plants and mosses in a old pasture, IX.–X.

Type revision. [*406* – PC GMC38] Basidiospores 5.7–9.1 × 4.8–6.7 µm, average 7.4 × 5.8 µm, E = 1.08–1.49, Q = 1.28, with coarse warts, moderately dense, isolated or round. Cheilocystidia not seen, it is not

possible to distinguish the lamellar edge with certainty. Pleurocystidia rare, fusiform macrocystidia, with pointed apex, usually devoid of crystals. Stipitipellis consisting of long and narrow hyphae, with rare cylindrical hairs, caulocystidia absent. Pileipellis not observable in the type specimen.

Remarks. According to Vizzini et al. (2011) M. *striimarginata* belongs to their subclade A3.1 comprising four acystidiate taxa, *M. striimarginata* Métrod ex Bon, *M. "paratristis," M. graminicola* (Velen.) Kühner & Maire, and *M. angelesiana* A.H. Sm. (= *M. monticola* here). However, their sequences of *M. paratristis* (MCVE12645, JF908357), *M. striimarginata* (ANC M0202, JN616468) and *M. graminicola* (ANC M0201, JN616438) group with *M. stridula*. Moreover, macrocystidioid pleurocystidia were found in the type species of *M. striimarginata*. Therefore, we consider this species an unclear taxon.

1. **Identification key to European species of the subgenus** Urticocystis

This key is based on Antonín et al. 2014, 2015, 2017, 2021 and this publication.

Remarks. The confirmation of the presence or absence of cheilocystidia is often rather difficult in species of subg. *Urticocystis*. If their presence is not seen in the first preparation, the microscopic observation should be to repeated with additional 4–5 preparations to sure, if cheilocystidia are present or absent. The best places for cheilocystidia study seem to be near the lamellae insertion to the stipe. The similar problems can appear with observation of pleurocystidia and also caulocystidia or caulobasidia at the stipe (caulohymenium). The presence/absence of caulobasidia is also depending on quality of drying process when caulobasidia may easily collapse. Some species have a typically large pileus, but basidiomata having smaller pilei sometimes occur depending on growing conditions. We strongly recommend to combine both morphological and molecular data to identify species within subg. *Urticocystis* properly.

1a. Stipe with distinct dark brown or black-brown squamules M. verrucipes

1b. Stipe without such dark squamules 2

2a. Cheilocystidia present, in the form of macrocystidia; lamellae cream ochraceous, ochraceous brown, orangish ochraceous or salmon orange

M. cognata (the only species of subg. *Urticocystis* with macrocystidia)

2b. Cheilocystidia present or absent, if present then only in the form of urticoid cystidia; lamellae with different colour 3

3a. Context in the stipe base white or whitish (rarely pale grey-whitish, brown-whitish or with fine orange-brown tinge in their outermost margin) 4

3b. Context in the stipe base differently coloured (orange, orange-brown, yellowish brown, rusty brown, brown, pale brown, dark brown, black-brown, grey, grey-brown, grey-blue, blue, violaceous) 14

4a. Pleurocystidia absent 5

4b. Pleurocystidia present 12

5a. Cheilocystidia absent 6

5b. Cheilocystidia present 9

6a. Caulocystidia absent M. paedida

6b. Caulocystidia present 7

7a. Pileus 20–50 mm broad; stipe 30–70 × 2–7 mm, cylindrical or only slightly broadened at base M. acystidiata

7b. Basidioma usually larger, pileus 25–175 mm broad and stipe 30–100 × 3–17 mm; stipe always broadened, clavate to distinctly bulbose at base 8

8a. Pileus 25–175 mm broad; stipe 30–80(–155) × 3–17 mm, surface rather distinctly longitudinally fibrillose or longitudinally striate, entirely (especially at apex), floccose M. monticola

8b. Pileus 30–85 mm broad; stipe 45–100 × 4.5–10 mm, only at apex finely floccose or pubescent M. brachyspora

9a. Stipe small 5–25 × 2.5–5 mm M. galbuserae

9b. Stipe larger (longer and/or wider) 10

10a. Basidioma robust, pileus 54–170(–260) mm broad, stipe width 7–15 mm M. grammopodia

10b. Basidioma smaller, pileus 20–75 mm broad, stipe width 2–10 mm 11

11a. Cheilocystidia 18–50 × 5–11 µm, scattered M. graminicola

11b. Cheilocystidia 50–74 × 5–10 µm, very numerous M. paedida

12a. Caulocystidia absent M. paedida

12b. Caulocystidia present 13

13a. Stipe 20–70 × 2.5–7 mm, caulobasidia present M. exscissa

13b. Stipe 50–60 × 7–11 mm, caulobasidia absent M. rasilis

14a. Context in the stipe base with blue, blue-grey (or violaceous) shades M. juliannae

14b. Context in the stipe base without blue, blue-grey (or violaceous) shades 15

15a. Purpurascens or red tinges at the stipe base M. rufipes

15b. Not such colours at the stipe base 16

16a. Margin of the pileus white M. luteolosperma

16b. Margin of the pileus not white 17

17a. Context in the stipe base pale yellowish/orange M. castaneofusca

17b. Context in the stipe base differently coloured (darker) 18

18a. Caulobasidia present 19

18b. Caulobasidia absent 20

19a. Mainly 4-spored, but also less frequent 2-spored, clavate caulobasidia present M. stepposa

19b. Mainly (always?) 2-spored, clavate caulobasidia present M. malenconii

20a. Cheilocystidia absent 21

20b. Cheilocystidia present 23

21a. Average length of basidiospores over 8.3 µm M. microcephala

21b. Average length of basidiospores under 8.3 µm 22

22a. Pileus uniformly rather dark (grey-)brown or dark brown, sometimes slightly darker at centre M. stridula

22b. Pileus whitish grey, grey-brown, dark brown, pallescent up to ochraceous brown to brown, margin paler than centre in young specimens M. fontenlae

23a. Caulocystidia absent 24

23b. Caulocystidia present 26

24a. Lamellae grey (greyish when young) M. tristis

24b. Lamellae white to whitish or cream coloured (sometimes with greyish reflex), then pale ochraceous or greyish, with beige reflex 25

25a. Spores (broadly) ellipsoid, verruculose, warts mostly irregularly shaped and sized, sometimes up to 0.75 µm high, sometimes with rare ridges M. fontenlae

25b. Spores ellipsoid, less frequently fusoid-ellipsoid, verruculose, warts isolated, rarely connected, up to 1.0 µm high M. graminicola

26a. Cheilocystidia mainly of the *exscissa*-type 27

26b. Cheilocystidia mainly of the *brevipes*-type 31

27a. Only other than urticoid caulocystidia present 28

27b. Urticoid caulocystidia present (but other type of caulocystidia may be also present) 29

28a. Stipe dark yellowish brown, basally blackish brown M. zaaminensis

28b. Stipe pale ochraceous (ochraceous-grey, grey) M. diverticulata

29a. Lamellae grey (greyish when young) M. tristis

29b. Lamellae white to whitish or cream-coloured (sometimes with greyish reflex), then pale ochraceous or greyish, with beige reflex 30

30a. Spores (broadly) ellipsoid, verruculose, warts mostly irregularly shaped and sized, sometimes up to 0.75 µm high, sometimes with rare ridges M. fontenlae

30b. Spores ellipsoid, less frequently fusoid-ellipsoid, verruculose, warts isolated, rarely connected, up to 1.0 µm high M. graminicola

31a. Urticoid caulocystidia absent M. zaaminensis

31b. Urticoid caulocystidia present (but other type of caulocystidia may be also present) 32

32a. Only urticoid caulocystidia present M. juliannae var. decolorans

32b. Urticoid caulocystidia and also other type of caulocystidia present 33

33a. Lamellae crowded, L = c. 70–80, l = 3–4 M. humilis

33b. Lamellae moderately close to rather close, L = c. 30–65, l = 2–5 34

34a. Pileus uniformly rather dark (grey-)brown, sometimes slightly darker at centre M. stridula

34b. Pileus whitish grey, grey-brown, dark brown, pallescent up to ochraceous brown to brown, margin paler than centre in young specimens 35

35a. Stipe brownish orange at apex, brown to dark brown towards base M. romanensis

35b. Stipe lustrous whitish to slightly brownish at apex, ochraceous yellowish to brownish at centre, dark grey-brown towards base M. fontenlae

Discussion

The taxonomic revision of European *Melanoleuca* subg. *Urticocystis* revealed several examples of taxonomic problems: A) Incorrectly used name because type specimens belong to another species. In such cases the new name should be proposed. This issue was revealed at *M. monticola* (up to now treated as American species *M. angelesiana*) in this study. *Melanoleuca fontenlae* (treated in past as *M. pseudopaedida* – the type of *M. pseudopaedida* is conspecific with *M. luteolosperma*) and *M. acystidiata* (treated as *M. robertiana* – the type of *M. robertiana* belongs to *M. pallidicutis* having macrocystidia) are another example of this phenomenon (Antonín et al. 2021, 2022). B) Phylogeneticaly different but morphologicaly and ecologicaly similar species across the continents deserve attention of fungal taxonomists – *M. acystidiata* has a North American sister species *M.* aff. *acystidiata.* In general, *Melanoleuca* diversity in North America deserve a separate study. C) Rather high variability in ITS sequences of several species (*M. castaneofusca, M. exscissa, M. luteolosperma, M. stridula*) which is not supported by the sequence variability of other genes (Antonín et al. 2017, 2021; this study). Special attention could be paid to *M. diverticulata – M. rasilis* group. Although the species have identical ITS and tef1 sequences, they differ in rpb2 (Antonín et al. 2017). The species are kept separately due to morphological differences, but the detailed study of more specimens the taxa is desirable.

During description of new species special attention should be paid on DNA sequence data processing, especially if the new species is based on a single collection: Recently proposed *Melanoleuca chalcibasis* Voto, Maraia & Milanese (Voto et al. 2021) was delimited due to isolated position of the sequences of the specimen in the ITS, tef1a and concatenated phylogentic trees. Anyway, after examination of the sequences of holotype specimen (MZ621144 – ITS, MZ962408 – tef1) kindly provided by the 1st author of the paper, the substantial errors were revealed: about 105 first nucleotide positions in ssu-ITS1 region are completely erroneous (Suppl. Figure 1) and therefore biased the phylogenetic analyses. If these erroneous nucletides are excluded, the ITS sequence undoubtly groups among *M. luteolosperma* specimens. Therefore *M. chalcibasis* is a synonym of *M. luteolosperma.* The similar but less serious problem of erroneous nucleotides (especially at the beginnings or ends of sequences) occur at tef1 sequence of this paper and several ITS sequences published by Vizzini et al. (2011).

During our previous studies (Antonín et al. 2014, 2015, 2017, 2021) the phylogenetic position of two remarkable species – *M. cognata* and *M. verrucipes* was recognized. Both species are morphologicaly easily distinguishable. *Melanoleuca verrucipes* is especially characterized by the presence of dark brown to black-brown squamules on the white stipe, which is a unique character in this genus. *Melanoleuca cognata* is distinguishable by its orange-yellow to darker brown pileus, orange-yellow to salmon buff coloured lamellae, an ochraceous brown stipe, and its occurrence in spring (Boekhout 1988). While *M. verrucipes* has a basal position of *M exscissa* group and is likely to be closely related to *M exscissa*, *M. cognata* has an isolated position between the two, *M exscissa* and *M. castaneofusca* groups but more closely related to the later (Fig. 1). The presence of macrocystidia at *M. cognata* obviously does not refer to relationship of this species to subg. *Melanoleuca*, where macrocystidia dominate in hymenium but is an apomorphic character of the species.

Declarations

Statements & Declarations

No potential conflict of interest was reported by the authors.

Funding

The studies of V.A. and H.Š. were made possible by the support provided to the Moravian Museum by the Ministry of Culture of the Czech Republic as part of its long-term conceptual development program for research institutions (DKRVO, ref. MK000094862). The research of T.K. and M. T. was supported by the Czech Ministry for Education, Youth and Sports and the European Regional Development Fund for financing the Project Phytophthora Research Centre Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000453. The research of O. Ď. was supported by VEGA: VEGA 1/0749/21.

Competing Interests

All authors declare they have no financial interests.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and data analysis were performed by Vladimír Antonín, Ondrej Ďuriška, Soňa Jančovičová, Tomáš Kudláček, Roberto Para, Hana Ševčíková and Michal Tomšovský. The figures were prepared by Vladimír Antonín, Soňa Jančovičová and Michal Tomšovský. The first draft of the manuscript was written by [Vladimír Antonín, Ondrej Ďuriška, Hana Ševčíková and Michal Tomšovský] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The data generated during and analysed during the current study are available in the NCBI repository.

Acknowledgements

We thank the curators of the ANC, H, LIP, MPU, PC, PRM, and SLO herbaria for loans of the type and other specimens, and S. Adamčík (Slovakia), M.T. Basso (Italy), M. Beran (Czech Republic), J. Borovička (Czech Republic), F.-X. Boutard (France), M. Caboň (Slovakia), R. Ejrnaes (Denmark), H. Forstinger (Austria), S. Glejdura (Slovakia), L. Hagara (Slovakia), A. Hausknecht (Austria), J. Holec (Czech Republic), H. Huijser (The Netherlands), D. Janda (Czech Republic), O. Jindřich (Czech Republic), V. Kautman (Slovakia), S. Komínková (Czech Republic), J. Novotný (Czech Republic), P. Kešeľák (Slovakia), E. Suarez (Spain), M. Vašutová (Czech Republic), and A. Verbeken (Belgium) for providing their collections for our studies and our herbaria. R.P. also thanks his friend Roberto Fontenla for collaboration in the study of this genus. The authors also wish to thank Roman Plichta (Mendel University in Brno, Czech Republic), for help with the SEM microphotography of the basidiospores. The studies of V.A. and H.Š. were made possible by the

support provided to the Moravian Museum by the Ministry of Culture of the Czech Republic as part of its long-term conceptual development program for research institutions (DKRVO, ref. MK000094862). The research of T.K. and M.T. was supported by the Czech Ministry for Education, Youth and Sports and the European Regional Development Fund for financing the Project Phytophthora Research Centre Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000453. The research of O.Ď. was supported by VEGA Scientific Grant Agency: VEGA 1/0749/21.

References

1. Antonín V, Ďuriška O, Jančovičová S, Tomšovský M (2015) Identity of *Agaricus brevipes* Bull. (*Melanoleuca brevipes*, Tricholomataceae, Basidiomycota). Mycol Progress 14:107. https://doi:10.1007/s11557-015-1125-0
2. Antonín V, Ďuriška O, Gafforov Y, Jančovičová S, Para R, Tomšovský M (2017) Molecular phylogenetics and taxonomy in *Melanoleuca* (*Tricholomataceae*, Basidiomycota) with emphasis on *M. exscissa* group and the description of *M. griseobrunnea* sp. nov. Pl Syst Evol 303:1181–1198. https://doi:10.1007/s00606-017-1430-y
3. Antonín V, Ďuriška O, Jančovičová S, Tomšovský M (2018) Two less known *Melanoleuca* species, *M. malenconii* and *M. tristis* (Basidiomycota, Agaricales) from anthropogenous habitats in the Czech and Slovak Republics. Acta Mus Moraviae, Sci Biol, 103:287–297
4. Antonín V, Ďuriška O, Para R, Ševčíková H, Tomšovský M (2021) *Melanoleuca galbuserae*, *M. fontenlae* and *M. acystidiata*, three new species in subgen. *Urticocystis*, and comments to *M. castaneofusca* and related species. J Fungi 7(3):191. https://doi.org/10.3390/jof7030191
5. Antonín V, Ďuriška O, Jančovičová S, Para R, Kudláček T, Tomšovský M (2022) Multilocus phylogeny and taxonomy of European *Melanoleuca* subgenus *Melanoleuca*. Mycologia 114(1):114–143. https://10.1080/00275514.2021.1966246
6. Antonín V, Rimóczi I, Benedek L, Papp V, Szarkándi JG, Dima B, Nagy LG, Papp T, Ďuriška O, Tomšovský M (2014) *Melanoleuca juliannae* (Basidiomycota, Tricholomataceae), a new species from subgen. Urticocystis Phytotaxa 170:13–23. https://doi:10.11646/phytotaxa.170.1.2
7. Atchadé YF, Roberts GO, Rosenthal JS (2011) Towards optimal scaling of metropolis-coupled Markov chain Monte Carlo. Statist Comput 21(4):555–568. https://doi:10.1007/s11222-010-9192-1
8. Boekhout T (1988) Notulae ad floram agaricinam neerlandicam—XVI. New taxa, new combinations in *Melanoleuca* Pat. and notes on rare species in the Netherlands. Persoonia 13:397–431
9. Boekhout T (1999) *Melanoleuca* Pat. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC (eds) Flora agaricina neerlandica, vol 4. AA Balkema, Rotterdam-Brookfield, pp 153–165
10. Bon M (1978) Taxon nouveaux. Doc Mycol 8(29):33–38
11. Bon M (1991) Les Tricholomes et ressemblant. Doc Mycol Mém Hors Sér 2:1–163
12. Bouckaert R, Drummond A (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evol Biol 17(1):42. https://DOI10.1186/s12862-017-0890-6

13. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C, Xie D, Suchard M, Rambaut A, Drummond A (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput Biol 10(4):e1003537. https://doi.org/10.1371/journal.pcbi.1003537
14. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. PLoS Biol 4:699–710. https://doi.org/10.1371/journal.pbio.0040088
15. Ďuriška O, Antonín V, Para R, Tomšovský M, Jančovičová S (2017) Taxonomy, ecology and distribution of *Melanoleuca strictipes* (Basidiomycota, Agaricales) in Europe. Czech Mycol 69:15–30. https://doi:10.33585/cmy.69102
16. Favre J (1948) Les associations fongiques des hauts-marais jurassiens. Matér Fl Cryptogam Suisse 10(3):9–228
17. Fontenla R, Gottardi M, Para R, Contu M (2003) Osservazioni sul genere *Melanoleuca*. Sesto contributo: neotipificazione di *Melanoleuca stridula*, una specie friesiana di interpretazione non univoca. Boll Gruppo Micol G. Bresadola, Nuova Ser, 46(2):31–39
18. He MQ, Zhao RL, Hyde KD, Begerow D, Kemler M, Yurkov A, McKenzie EHC, Raspé O, Makoto K, SánchezRamírez S, Vellinga EC, Halling R, Papp V, Zmitrovich IV, Buyck B, Ertz D, Wijayawardene NN, Bao-Kai C, Schoutteten N, Liu XZ, Li TH, Yao YJ, Zhu XY, Liu AQ, Li GJ, Zhang MZ, Ling ZL, Cao B, Antonín V, Boekhout T, da Silva BDB, De Crop E, Decock C, Dima B, Dutta AK, Fell JW, Geml J, Ghobad-Nejhad M, Giachini AJ, Gibertoni TB, Gorjón SP, Haelewaters D, He SH, Hodkinson BP, Horak E, Hoshino T, Justo A, Lim YW, Menolli N Jr, Mešić A, Moncalvo JM, Mueller GM, Nagy LG, Nilsson RH, Noordeloos M, Nuytinck J, Orihara T, Ratchadawan C, Rajchenberg M, Silva-Filho AGS, Sulzbacher MA, Tkalčec Z, Valenzuela R, Verbeken A, Vizzini A, Wartchow F, Wei TZ, Weiß M, Zhao CL, Kirk PM (2019) Notes, outline and divergence times of Basidiomycota. Fungal Divers 99:105–367. https://doi:10.1007/s13225-019-00435-4
19. Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinf 9:286–298. https://doi:10.1093/bib/bbn013
20. Kone A, Kofke DA (2005) Selection of temperature intervals for parallel-tempering simulations. J Chem Phys 122(20):1–2. https://doi:10.1063/1.1917749
21. Kornerup A, Wanscher JH (1983) Methuen handbook of colour, vol 3. Eyre Methuen, London
22. Kozlov A, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35(21):4453–4455. https://doi.org/10.1093/bioinformatics/btz305
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://DOI:10.1093/molbev/msy096
24. Kühner R (1978) Agaricales de la zone alpine. Genre Melanoleuca Pat Bull Soc Linn Lyon 47:12–52
25. Küppers H (2007) DuMont Farbenatlas. Rasch, Bramsche
26. Lanfear R, Frandsen P, Wright A, Senfeld T, Calcott B (2016) PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses.

Mol Biol Evol 34(3):772–773. https://doi.org/10.1093/molbev/msw260

27. Lange JE (1933) Studies in the Agarics of Denmark. Part IX. *Tricholoma*, *Lentinus*, *Panus*. *Nyctalis*. Dansk Bot Ark 8:1–46
28. Lange JE (1935) Flora agaricina Danica. Recato, Copenhagen
29. Lemoine F, Domelevo Entfellner J, Wilkinson E, Correia D, Dávila Felipe M, De Oliveira T, Gascuel O (2018) Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature 556(7702):452–456. https://doi.org/10.1038/s41586-018-0043-0
30. Malençon G, Bertault R (1975) Flore des champignons supérieurs du Maroc. Tome II. Trav Inst Sci Chérif Fac Sci Rabat 33:1–540
31. Métrod G (1948) Essai sur le genre *Melanoleuca* Patouillard emend. Bull Soc Mycol France 64:141–165
32. Müller N, Bouckaert R (2019) Adaptive parallel tempering for BEAST 2. bioRxiv [online]. https://Doi. 10.1101/603514
33. Osmundson TW, Robert VA, Schoch CL, Baker LJ, Smith A, Robich G, Mizzan L, Garbelotto MM (2013) Filling gaps in biodiversity knowledge for macrofungi: Contributions and assessment of an herbarium collection DNA barcode sequencing project. PLoS ONE 8:e62419. https://doi.org/10.1371/journal.pone.0062419
34. Pattengale N, Alipour M, Bininda-Emonds O, Moret B, Stamatakis A (2010) How many bootstrap replicates are necessary? J Comput Biol 17(3):337–354. https://DOI:10.1089/cmb.2009.0179
35. Rambaut A, Drummond A, Xie D, Baele G, Suchard M (2018) Posterior summarization in Bayesian phylogenetics using tracer 1.7. Syst Biol 67(5):901–904. doi: 10.1093/sysbio/syy032
36. Sánchez-García M, Cifuentes-Blanco J, Matheny PB (2013) Revisión taxonómica del género *Melanoleuca* en México y descripción de especies nuevas. Rev Mex Biodivers 84:111–127. https://doi.org/10.7550/rmb.31569
37. Singer R (1986) The Agaricales in modern taxonomy, 4th edn. Koeltz Scientific Books, Koenigstein
38. Sukumaran J, Holder MT (2010) DendroPy: A Python library for phylogenetic computing. Bioinformatics 26:1569–1571. https://DOI:10.1093/bioinformatice/btq228
39. Šutara J (2005) Central European genera of the Boletaceae and Suillaceae, with notes on their anatomical characters. Czech Mycol 57:1–50. https://doi:10.33585/cmy.57101
40. Thiers B (2022) continuously updated. Index Herbariorum: a global direktory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. available online at: http://sweetgum.nybg.org/ih/. Accessed 10 October 2022
41. Vesterholt J (2012) *Melanoleuca* Pat. In: Knudsen H, Vesterholt J (eds) Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp, Copenhagen, pp 347–352
42. Vizzini A, Para R, Fontenla R, Ghignone S, Ercole E (2011) A preliminary ITS phylogeny of *Melanoleuca* (Agaricales) with special reference to European taxa. Mycotaxon 118:361–381. https://doi:10.5248/118.361

43. Voto P, Maraia G, Milanese V (2021) *Melanoleuca chalcibasis* sp. nov. from the Bosco Nordio (Italy). Mycol Obs 1:35–43
44. Yu XD, Lv SX, Ma D, Li FF, Lin Y, Zhang L (2014) Two new species of *Melanoleuca* (Agaricales, Basidiomycota) from northeastern China, supported by morphological and molecular data. Mycoscience 55:456–461. https://doi:10.1016/j.myc.. 2014.01.007

Figures

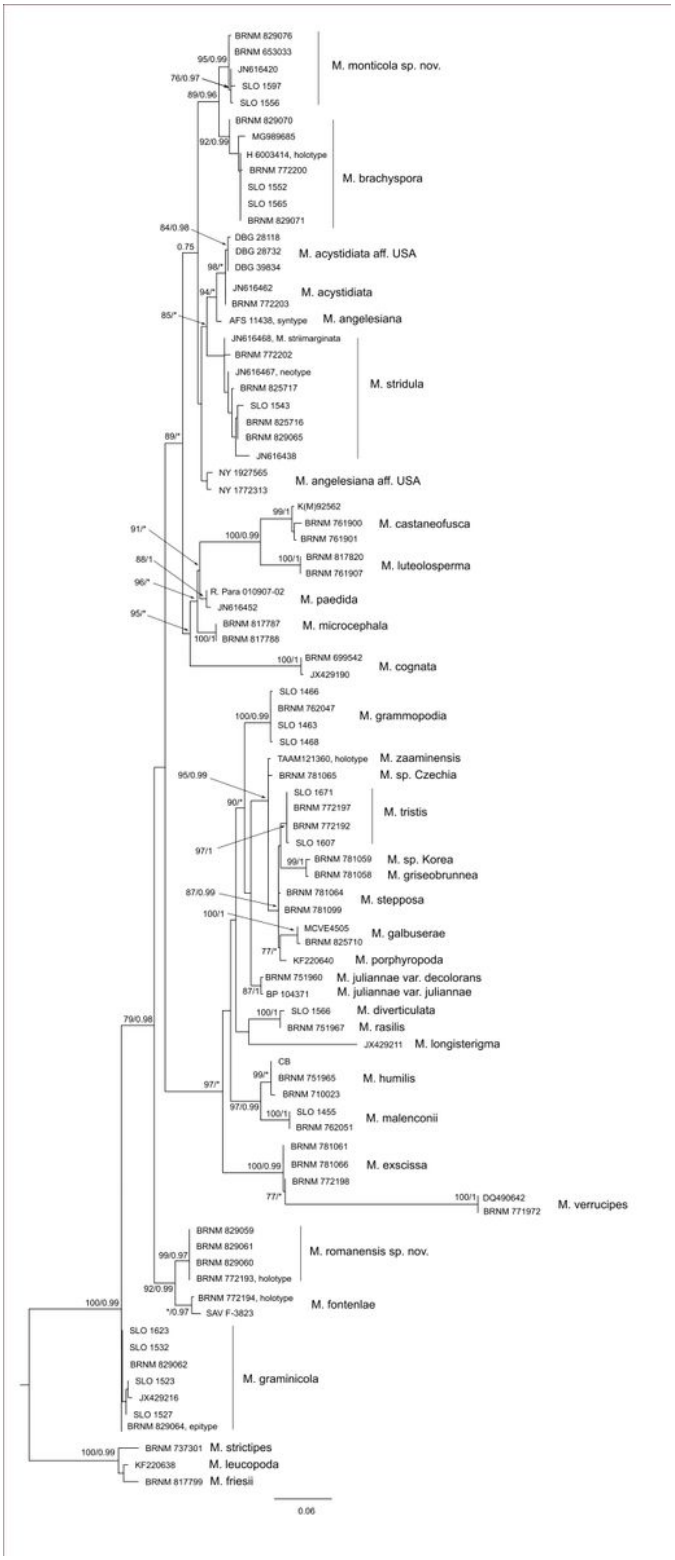

Figure 1

The phylogenetic tree of the ITS region based on Maximum Likelihood analysis (for legend to specimen numbers, see TABLE 1). Numbers at branches indicate maximum likelihood bootstrap support and Bayesian posterior probability values. The asterisks (*) mark low support (<75 in maximum likelihood; <90 in Bayesian analysis). The bar indicates the number of expected substitutions per position.

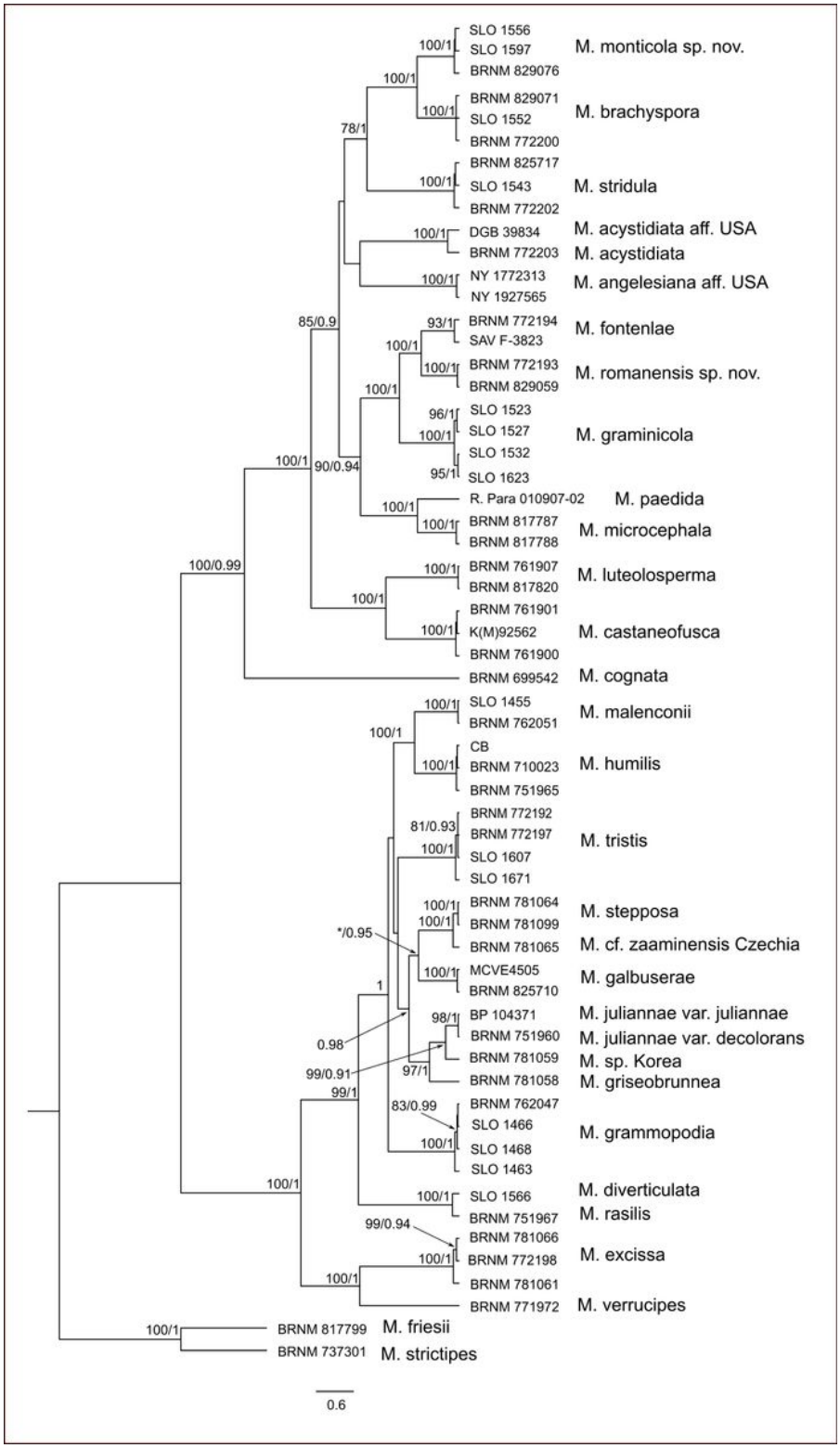

Figure 2

The phylogenetic tree of ITS-rpb2-tef1 regions based on Bayesian inference (for legend to specimen numbers, see TABLE 1). Numbers at branches indicate maximum likelihood bootstrap support and Bayesian posterior probability values. The bar indicates the number of expected substitutions per position.

Figure 3

Basidiomata of *Melanoleuca monticola* sp. nov. a (SLO1556, holotype), b (SLO1558), c (SLO1550), d (SLO1559), e (SLO1553), f (BRNM 829076).

Figure 4

Basidiomata of *Melanoleuca graminicola*. a (BRNM 829064, epitype), b (SLO1531), c (SLO1619), d (SLO1626); basidiomata of *Melanoleuca romanensis* sp. nov. e and f (BRNM 829059).

Figure 5

Melanoleuca brachyspora. a (PRM 958041), b (BRNM 772200), c (SLO1554), d (SLO1564); basidiomata of *Melanoleuca stridula*. e (BRNM 825717), f (SLO1557).

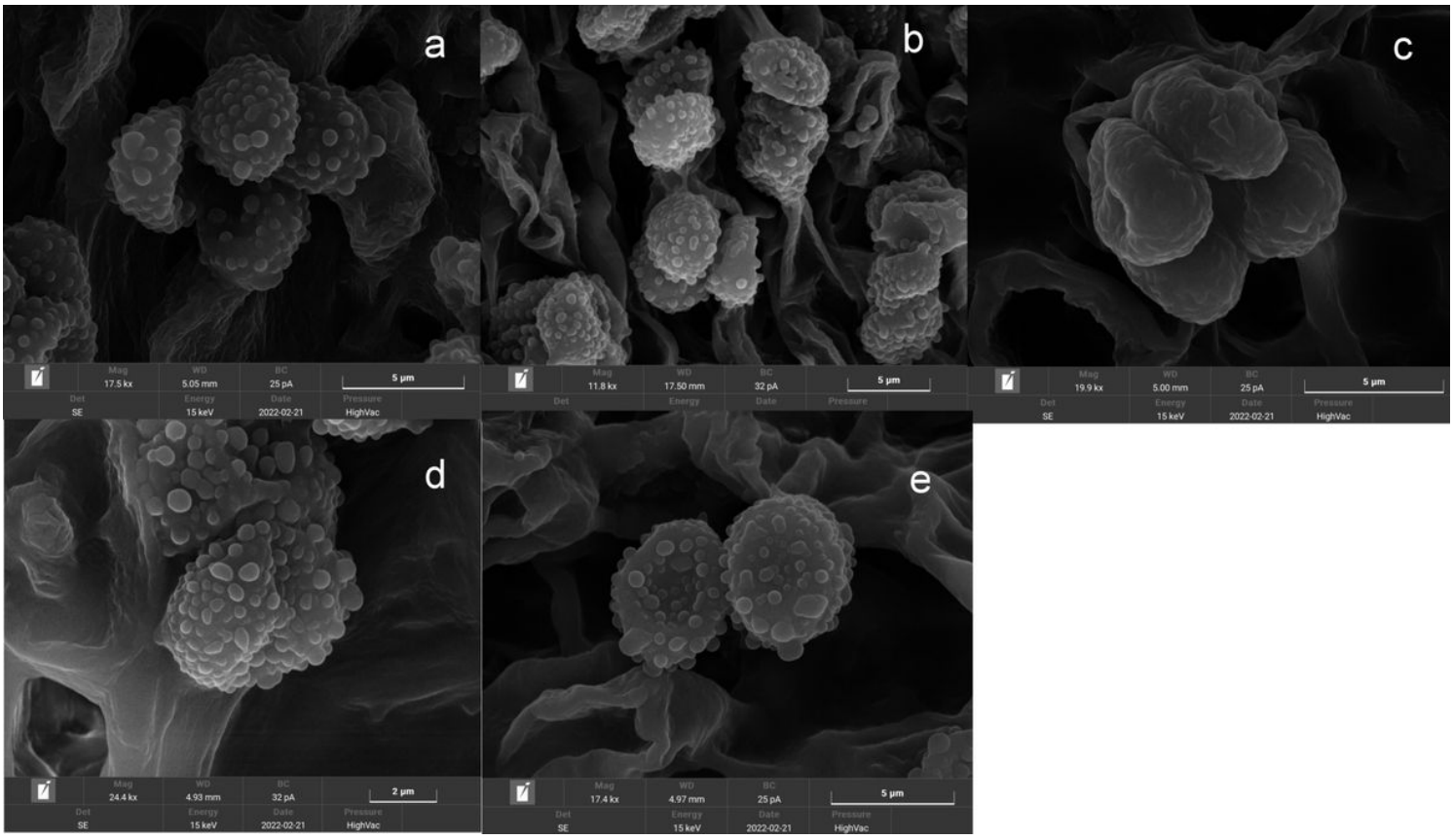

Figure 6

SEM microphotographs of *Melanoleuca*basidiospores. a. *M. brachyspora* (BRNM 829070), b. *M. graminicola* (BRNM 829062), c. *M. monticola* (BRNM 653033), d. *M. romanensis* (BRNM 829060), e. *M. stridula* (BRNM 829065). Photo R. Plichta and M. Tomšovský.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AntoninetalMelanoleucaSupplFig1.pdf