

**Two lesser-known *Melanoleuca* species, *M. malenconii* and *M. tristis*
(Basidiomycota, Agaricales) from anthropogenous habitats
in the Czech and Slovak Republics**VLADIMÍR ANTONÍN¹, ONDREJ ĎURIŠKA²,
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ANTONÍN V., ĎURIŠKA O., JANČOVIČOVÁ S. & TOMŠOVSKÝ M. 2018: Two lesser-known *Melanoleuca* species, *M. malenconii* and *M. tristis* (Basidiomycota, Agaricales) from anthropogenous habitats in the Czech and Slovak Republics. *Acta Musei Moraviae, Scientiae biologicae* **103(2)**: 287–297. – Two rare *Melanoleuca* species, *M. malenconii* and *M. tristis*, are published from the Czech and Slovak Republics. Their macro- and microscopic descriptions, based on own collections, are given, and the ecology and distribution of the species in these countries, as well as elsewhere in Europe, are summarized. Both species grow in a variety of habitats influenced by human activity. Both morphological and phylogenetic approaches are used to support species identification.

Key words. *Melanoleuca malenconii*, *Melanoleuca tristis*, Czech Republic, Slovakia, ecology, distribution

Introduction

The genus *Melanoleuca* Pat. is a cosmopolitan basidiomycetous agaricoid genus, currently included in suborder Pluteineae (DENTINGER *et al.* 2015), which contains more than 400 named species (<http://www.indexfungorum.org>). It has the following main characters: collybioid to tricholomatoid basidiomata, a convex to slightly depressed pileus, often with a low central umbo, emarginate, adnate to shortly (with tooth) decurrent lamellae, pileipellis and cutis, an ixocutis or a trichoderm, amyloid ornamented basidiospores, present or absent cheilocystidia and pleurocystidia and lack of clamp connections (e.g., BOEKHOUT 1988, 1999, BON 1991, KÜHNER 1978, SINGER 1986). This genus is easily identifiable at the generic level; however, the identification of individual species is rendered problematic by overlapping morphological characters in many species. The use of phylogenetic methods is required for the identification of many taxa (e.g., ANTONÍN *et al.* 2014, 2015, 2017, VIZZINI *et al.* 2011, YU *et al.* 2014).

Both studied species possess urticoid cheilocystidia, and therefore belong to the *Melanoleuca* subgenus *Urticocystis* Boekhout (BOEKHOUT 1988). Several species groups of this subgenus have been already taxonomically and phylogenetically addressed by e.g., ANTONÍN *et al.* (2014, 2015, 2017) and VIZZINI *et al.* (2011).

Material and methods

Macroscopic descriptions are based on fresh basidiomata collected during recent field work. The authors of fungal names are cited according to the *Authors of Fungal Names* page (<http://www.indexfungorum.org/AuthorsOfFungalNames.htm>); colour abbreviations follow KORNERUP & WANSCHER (1983), and herbarium abbreviations follow THIERS (2018). Microscopic features are described from dried material mounted in KOH, Melzer's reagent, and Congo Red, using an Olympus BX-50 light microscope (Tokyo, Japan) at a magnification of 1000×. Microscopic description is based on 30 measurements of basidiospores, and 10 measurements of basidia, cheilocystidia and caulocystidia of each specimen (in some cases, cheilocystidia, or some caulohyphenium cells resp., were so rare that fewer than 10 measurements were possible). For lamellae, L is the number of all lamellae of one basidioma. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (range of means of E-values) are used. Characters of cheilocystidia and pleurocystidia are defined according to VIZZINI *et al.* (2011) – either with a narrow, cylindrical upper part (the *brevipes*-type), or with a rather wide upper part attenuating towards the apex (the *excisssa*-type). Microcharacter drawings were processed in the CorelDraw programme.

The species were subject to molecular phylogenetic analyses which confirmed their identity and position within the genus (ANTONÍN *et al.* 2015, 2017). The GenBank accession numbers of our sequences are given in brackets after herbarium specimen numbers in the “Specimens examined” section.

Species descriptions

***Melanoleuca malenconii* Bon, Documents Mycologiques 20(79): 59, 1990**
(Figs 1–4)

= *Melanoleuca sublanipes* Fontenla, Para & Vizzini, Mycotaxon 118: 373, 2011.

= *Melanoleuca turrata* s. Malençon (FONTENLA *et al.* 2003, MALENÇON & BERTAULT 1975).

Description. Pileus 20–70 mm broad, plano-convex, then applanate, centrally slightly depressed and/or slightly umbonate, margin reflexed, then ± straight, not translucently striate, surface slightly pubescent (especially at margin) when young, later ± glabrous, in some collections apparently brown-black tomentose at centre, uniformly coloured, grey, ochraceous to dirty yellow (4–6B3–4, 6C1–2, 7D2) or with dark brown (7E3–4, 7E–F8) centre. Lamellae moderately close, L = 50–60, emarginate and adnate with tooth, white to cream, later up to pale to greyish yellow (up to 4A–B3), edge concolorous. Stipe 22–50×3–6(–7) mm, cylindrical, slightly broadened at apex, ± clavate to bulbous (up to 18 mm broad) at base, sometimes laterally compressed, longitudinally fibrillose, slightly pruinose-pubescent to distinctly floccose, rarely subglabrous at apex, sometimes especially in lower part floccose-hairy, grey-brown (6E–F3–4, 7D3, 7E4), with paler apex when young. Context whitish to pale brownish in pileus, dirty grey-brown, dark brown or rusty brown in stipe base; lacking distinct smell or with slightly fungoid odour and with mild or bitterish taste.



Fig 1. *Melanoleuca malenconii*. Basidiomata (Czech Republic, Roudnice nad Labem, Kleneč, BRNM 762051). Photo D. Marounek.

Basidiospores $(6.0-7.4-10 \times 4.5-5.0-6.0(-7.0) \mu\text{m}$, average = $8.8 \times 5.7 \mu\text{m}$, $E = (1.07-1.42-1.82(-2.0)$, $Q = 1.37-1.59$, (broadly) ellipsoid or ovoid, verruculose with small ridges and (less frequent) connections, amyloid. Basidia $(30-32-40 \times 8.0-9.0-13 \mu\text{m}$, 4-spored, clavate. Cheilocystidia urticoid, of both the brevipes-type and excissa-type, $22-60 \times 3.0-14 \mu\text{m}$, rostrum $(1.5-2.75-5.0 \mu\text{m}$ wide, basal part fusoid to subcylindrical, thin-walled, apex subulate or cylindrical, obtuse, thin- to slightly thick-walled, with or without apical crystals. Marginal cells $26-32 \times 5.5-10 \mu\text{m}$, clavate, fusoid, irregular or with projection, thin-walled. Pleurocystidia similar to cheilocystidia, sometimes absent. Pileipellis an ixocutis to an ixotrichoderm (margin), composed of \pm cylindrical, \pm thin-walled, non-dextrinoid, up to $8.0(-10) \mu\text{m}$ -wide hyphae; terminal cells up to $60 \times 5.0-10 \mu\text{m}$, appressed to erect, cylindrical, narrowly clavate, subfusoid, obtuse. Stipitipellis a cutis of cylindrical, parallel, \pm thin-walled, non-dextrinoid, up to $6.0(-8.0) \mu\text{m}$ -wide hyphae. Caulohymenium of three cell types: (1) $16-50 \times 3.0-15 \mu\text{m}$, clavate or cylindrical, thin-walled cells, (2) 2-spored, probably rarely also 4-spored, clavate basidia, and (3) urticoid cystidia, of the excissa-type or \pm brevipes-type, similar to cheilocystidia, $24-60 \times 5.5-9.0(-12) \mu\text{m}$, rostrum $3.0-5.0 \mu\text{m}$ wide; cystidia and caulobasidia sometimes very rare (or even absent).



Fig 2. *Melanoleuca malenconii*. Basidiomata (Czech Republic, Roudnice nad Labem, Kleneč, BRNM 762051). Photo D. Marounek.



Fig 3. *Melanoleuca malenconii*. Basidiomata (Italy, Ravenna, Marina di Ravenna, BRNM 761883). Photo V. Antonín.

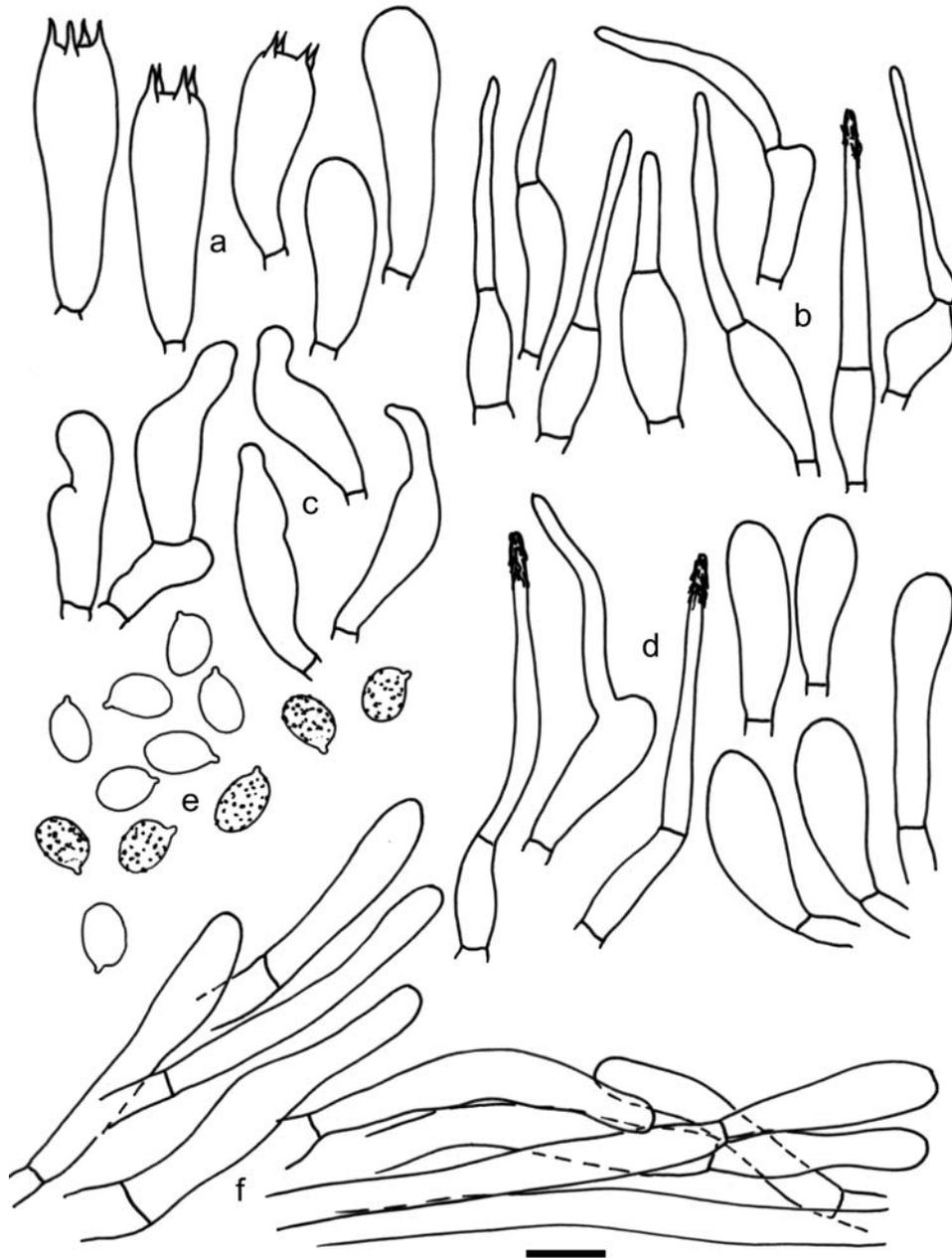


Fig 4. *Melanoleuca malenconii*. Microscopic characters: a. basidia and basidiola, b. cheilocystidia, c. marginal cells, d. caulohymenium, e. basidiospores (all basidiospores are ornamented), f. pileipellis. Scale bar = 10 μ m. Del. S. Jančovičová and V. Antonín.

Ecology and distribution. *Melanoleuca malenconii* grows on soil and appears to prefer various anthropogenically-influenced habitats. It can be found in both non-forest habitats (grasslands), e.g. lawns, gardens, courtyards, grass verges of roads, meadows (e.g., Vizzini *et al.* 2012, our collections), and (often artificial) forest stands on sandy soils and sandy dunes composed of e.g. *Pinus maritima*, *P. pinea*, and *Quercus ilex*, (e.g., Bon 1990, 1991, our collections).

Collections of this species have been confirmed from the Czech Republic, France, Italy, Slovakia and Spain, but it is probably widely distributed throughout Europe.

Specimens examined. **Czech Republic:** Roudnice nad Labem, Kleneč, 6 Dec. 2008 leg. J. Borovička, M. Kříž & D. Marounek (BRNM 762051; KP192275, KT279053 and KT279062). Brno, courtyard of the Moravian Museum, 5 Nov. 2009 leg. V. Antonín 09.357 (BRNM 762046; KP192276). **France:** Lieu La Grande Motte (34), 15 Nov. 1985 leg. G. Chevassut (Bon 3474, LIP!), as *M. turrita*, holotype of *M. malenconii*; KP192294). Wingles, Pas-de-Calais, 17 Oct. 1990 leg. R. Courtecuisse (LIP, RC/F90.058, as *M. brevipes*, KP192295). **Italy:** Veneto, Padova, Montegrotto Terme, 7 Nov. 2008 leg. G. Zecchin (ANC M0222!, holotype of *M. sublanipes*). Emilia-Romagna Prov., Ravenna, Lido di Dante, 9 Nov. 2000 leg. V. Antonín 00.229 (BRNM 762049). Emilia-Romagna Prov., Ravenna, Marina di Ravenna, 5 Nov. 2007 leg. V. Antonín 07.419 & M. Marcheggiani (BRNM 761883). **Slovakia:** Podunajská nížina, Bratislava, Apollo business centre, 24 Oct. 2010 leg. V. Zářecká (SLO 1451). Podunajská nížina, Bratislava, Petržalka, 30 Oct. 2012 leg. Čáčaná (SLO 1452). Podunajská nížina, Bratislava, Botanical garden, 16 Nov. 2012 leg. O. Ďuriška (SLO 1455; KP192277, KT279054 and KT279063); *Ibid.*, 16 Nov. 2012 leg. O. Ďuriška (SLO 1457). Podunajská nížina, Bratislava, Airport, 3 Oct. 2012 leg. L. Pomšár (SLO 1458). Malé Karpaty, Bratislava, 8 Sept. 2012 leg. D. Ďuriška (SLO 1456; KP192278). Malé Karpaty, Bratislava, Krasňany, 12 Nov. 2012 leg. I. Kautmanová (SLO 1454). Západné Beskydy, Slanická osada, 11 Oct. 2012 leg. O. Ďuriška (SLO 1453; KP192279). **Spain:** Teruel, 26. Oct. 2015 leg. E. Suarez (herb. Suarez).

Melanoleuca tristis M.M. Moser, *Boletus* 15: 66, 1991 (Figs 5–6)

Description. Pileus 30–65 mm broad, applanate or broadly funnel-shaped, with low, obtuse central umbo, with involute, then straight or slightly inflexed, irregular to undulate, not striate or striate to sulcate (up to *c.* 5 mm) margin, smooth, glabrous, slightly slippery, dark brown (7F6–7, 6F8, 8F5) when moist, pallescent to brown (7D4–5, 7E5–6) when drying out. Lamellae quite close, L = *c.* 55–70, emarginate, attached to shortly decurrent with tooth, sinuate, quite narrow (up to 6 mm), greyish, then distinctly grey (6D3, 7D2 at base, up to 7D–E3 towards edge), with concolorous edge. Stipe 22–55×3.5–7 mm, cylindrical, slightly broadened at apex, cylindrical or clavate (up to 13 mm) at base, longitudinally coarsely fibrillose, finely pubescent, especially at apex, ± uniformly dark grey-brown or black-brown to dirty dark brown (6E–F3, 8F4–5). Context dirty brownish, dark brown in stipe cortex, up to dark brown (6–7E8) in stipe base, lacking smell or with sweetish odour (reminiscent of baked potatoes or rubber) and mild taste.

Basidiospores 7.0–10×5.0–6.0(–6.5) μm, average 8.4×5.5 μm, E = 1.27–1.80, Q = 1.39–1.60, (broadly) ellipsoid, ornamentation verruculose, warts irregular, variable in shape and size, rarely with ridges, up to 0.75 μm high, amyloid. Basidia 24–48×8.0–13 μm, 4-spored, clavate, subutriform or subfusoid. Cheilocystidia urticoid of the excisssa-type, sometimes also brevipes-type, 30–56×5.0–11 μm, basal part ± fusoid, thin-walled, apical part subulate to (sub)cylindrical, with or without apical crystals. Pleurocystidia



Fig 5. *Melanoleuca tristis*. Basidiomata (Italy, Ravenna, Pineta di San Vitale, Bardello, BRNM 772192). Photo V. Antonín.

scattered, similar to cheilocystidia, sometimes absent. Marginal cells $15\text{--}33 \times 6.0\text{--}11\text{--}(15)$ μm , clavate, cylindrical, utriform, subfusoid, usually irregular or rarely branched, thin-walled. Pileipellis an ixocutis with transitions to an ixotrichoderm (centre) composed of cylindrical, thin-walled, non-dextrinoid, up to 9.0 μm -wide hyphae; terminal cells adpressed to erect, cylindrical, clavate, subfusoid, obtuse, thin-walled, $4.0\text{--}12$ μm wide, grey or brownish-grey in KOH. Stipitipellis a cutis of cylindrical, parallel, \pm slightly thick-walled, non-dextrinoid, up to 7.0 μm wide hyphae. Caulohymenium of two cell types: (1) $17\text{--}32 \times 10\text{--}15$ μm , clavate, fusoid, subcylindrical, thin-walled, and (2) $35\text{--}45 \times 7.0\text{--}15$ μm , urticoid cystidia, thin-walled; sometimes cystidia of type (2) may be sometimes very rare, or even absent.

Ecology and distribution. All the collections of *Melanoleuca tristis* studied are known from habitats influenced by human activity. The fungus grows on soil under *Pinus sylvestris* on serpentinite soil (holotype), in grasslands on fixed dunes (BRNM 772192, BRNM 772195 and BRNM 772199), and on anthropogenous sites in grass (BRNM 772197, SLO1607, SLO1671).

This species has been definitely recorded from the Czech Republic, Italy, Serbia and Slovakia (ANTONÍN *et al.* 2017). Collections from Austria are included in the Virtual herbarium WU (<http://herbarium.univie.ac.at/index.htm>), but these are not revised herein. However, it is probably widely distributed in central, southern and western Europe, but constitutes a rare species in all regions.

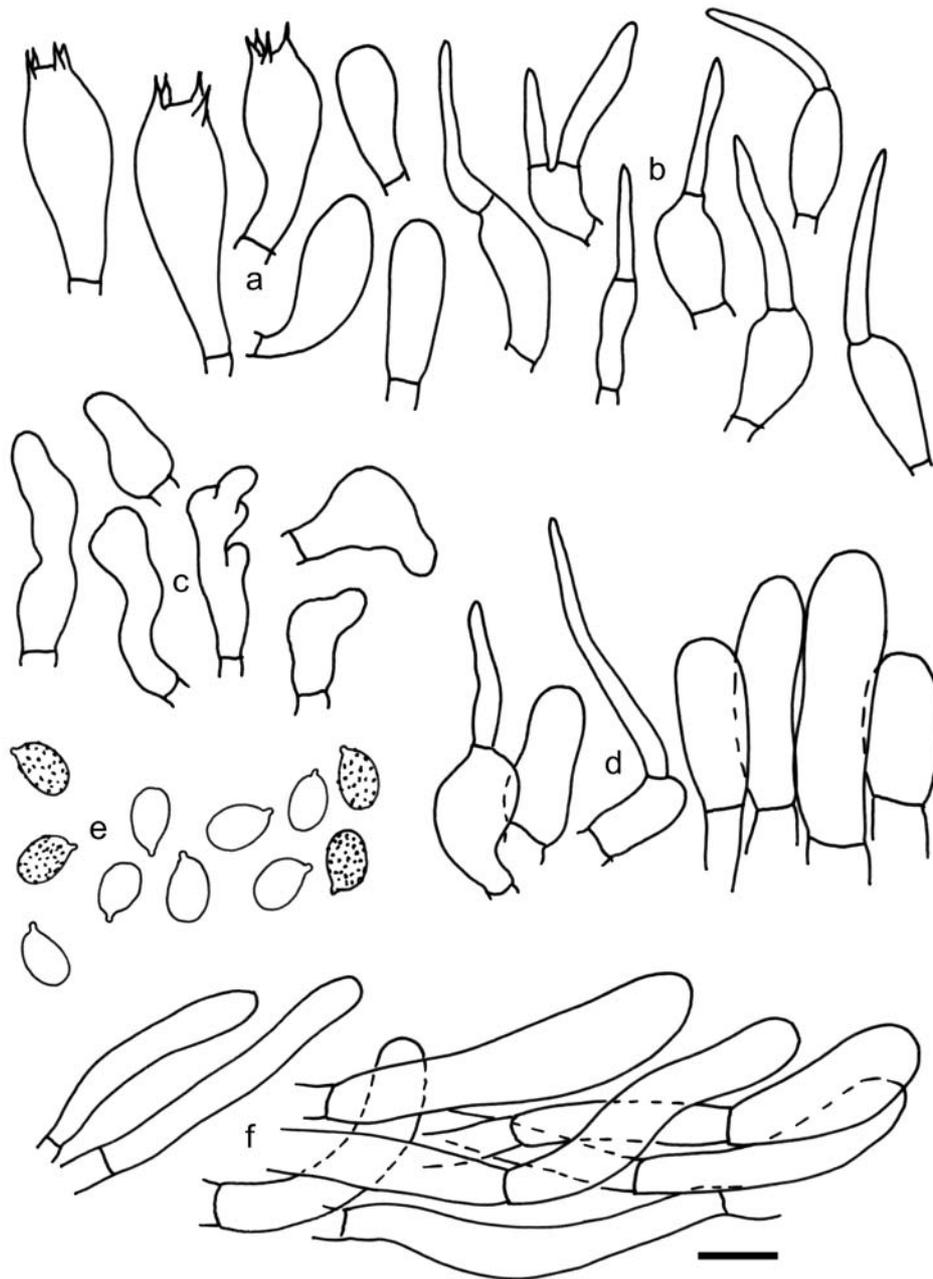


Fig 6. *Melanoleuca tristis*. Microscopic characters: a. basidia and basidiola, b. cheilocystidia, c. marginal cells, d. caulohymenium, e. basidiospores (all basidiospores are ornamented), f. pileipellis. Scale bar = 10 μ m. Del. S. Jančovičová and V. Antonín.

Specimens examined. **Czech Republic:** Třeboň, Botanical Institute, 29 Sept. 2005 leg. M. Vašutová (Antonín 05.188; BRNM 772197; LT594137, LT594168 and LT594184). **Italy:** Emilia-Romagna Prov., Ravenna Distr., Pineta di San Vitale, Bardello, 0–5 m a.s.l., 6 Nov. 2000 leg. V. Antonín 00.207 (BRNM 772192; LT594135, LT594167 and LT594183). Emilia-Romagna Prov., Ravenna Distr., Pineta di San Vitale, Bardello, 0–5 m a.s.l., 6 Nov. 2000 leg. M. Enderle (Antonín 00.255; BRNM 772195; LT594136). Emilia-Romagna Prov., Ravenna Distr., Pineta di San Vitale, Bardello, Lato Bedalassona-Rivalone, 6 m a.s.l., 8 Nov. 2007 leg. V. Antonín 07.438 (BRNM 772199; LT594138). **Serbia:** Tara Mts., Kaludjerske bare (as Kaludjarskoe Bare at label), c. 1000–1050 m a.s.l., 10 Oct. 1963 leg. M.M. Moser 1963/0722; KP192280 (IB s.n.!, holotype). **Slovakia:** Záhorská nížina, Lakšárská Nová Ves, Šišuláky, 23 Sept. 2013 leg. J. Kautman (SLO 1607; LT594139, LT594169 and LT594185). Záhorská nížina, Šaštín, Gazárka, 2 Nov. 2014 leg. I. Tomašková (SLO 1671; LT594140, LT594170 and LT594186).

Discussion

Melanoleuca malenconii is characterised by the following: pileus grey, ochraceous to dirty yellow, slightly pubescent (especially at margin), later ± glabrous, lamellae white to cream, then pale to greyish-yellow, stipe quite uniformly grey-brown, slightly pruinose-pubescent to distinctly floccose at apex, sometimes (especially in lower part) floccose-hairy, context brown to grey-brown in the stipe base, cheilocystidia urticoid, pleurocystidia often developed and similar to cheilocystidia, pileipellis in the form of an ixocutis or an ixotrichoderm, and the presence of a caulohymenium.

Melanoleuca malenconii has been collected with certainty in only a few countries in central and southern Europe, but it is probably widely distributed.

In the Czech Republic, *M. malenconii* is known from two localities (one from Bohemia, one from Moravia) and in Slovakia from eight localities (mostly in its south-western part) to date. It has always been found in artificial grassy swards in cities and villages, in gardens, along paths and on mulch bark.

In comparison with similar taxa, *Melanoleuca humilis* (Pers.) Pat. has a ± smooth, paler, grey-brown pileus, greyish to beige-coloured lamellae, a more robust (22–65×5–10 mm) stipe, which is paler coloured (dark watery-brown when moist, greyish when dried out) and an (orangish) brown context in the stipe base; *M. grammopodia* (Bull.: Fr.) Pat. differs in its more robust basidiomata (pileus 70–125 mm broad, stipe 17–110×7–15 mm), a greyish, whitish or brownish-tinged pileus with a grey-brown centre, cream or greyish beige lamellae, a whitish stipe base context, and caulocystidia of two types (ANTONÍN *et al.* 2015); *M. juliannae* Rimóczi *et al.* var. *decolorans* Antonín & Tomšovský has a pileus only 25–35 mm broad, smooth, glabrous, entirely dark brown, later becoming (grey-)brown from margin, an only slightly clavate-bulbose stipe (up to 7 mm wide at base), which is only finely pruinose-floccose at apex, no pleurocystidia and a caulohymenium of two types of cells (ANTONÍN *et al.* 2014). All the taxa mentioned also differ genetically from *M. malenconii* and form well-supported separated phylogenetic lineages (ANTONÍN *et al.* 2015, 2017).

Melanoleuca tristis is characterized by: pileus dark brown, lamellae greyish, stipe longitudinally distinctly fibrillose, finely pubescent especially at apex, uniformly dark grey-brown, black-brown to dark brown, context dark brown in stipe base, cheilocystidia urticoid, pileipellis in the form of an ixocutis transient to an ixotrichoderm at centre, and a developed caulohymenium.

It is a rare species in Europe, not included in most recent main identification keys (BOEKHOUT 1999, VESTERHOLT 2012, WATLING & TURNBULL 1998). We assume that it is more widely distributed in Europe, but it is certainly rare everywhere.

To date, *Melanoleuca tristis* is known from a single locality in the Czech Republic (in Bohemia) and from only two localities in south-western Slovakia. All these collections are from anthropogenous habitats. In Bohemia, it was found on an open site among greenhouses, and in Slovakia on a ruderalized site at a forest edge and on soil along a pathway.

Among similar taxa, *Melanoleuca malenconii* has a grey, ochraceous to dirty yellow, sometimes at centre brown, pileus, white to cream, then pale to greyish-yellow lamellae, and an often more distinctly floccose stipe, especially at apex; *M. rasilis* (Fr.) Singer differs in its larger, 40–100 mm broad, black-brown to almost black, in some places dark brown pileus, pale greyish to light brown lamellae and a more robust, 50–60×7–11 mm large stipe, smaller cheilocystidia (26–45×6.0–7.0 µm), and only one type of caulocystidia (clavate) (ANTONÍN *et al.* 2017, MOSER 1991); *M. humilis* has a paler, grey-brown pileus and a more robust, 22–65×5–10 mm large stipe, which is paler coloured, dark watery-brown when moist, greyish when dried out, and an (orange) brown context in the stipe base; *M. grammopodia* differs in its larger, 70–125 mm broad, greyish, whitish or brownish-tinged pileus, with a grey-brown centre, cream or greyish beige lamellae, a larger, 17–110×7–15 mm, grey-brown stipe, and a whitish stipe base context (ANTONÍN *et al.* 2015); *M. excissa* (Fr.: Fr.) Singer has a larger, 25–75 mm broad, grey-brown, beige-grey pileus with more distinctly grey centre, cream-coloured lamellae, sometimes with an orangish tinge, a whitish, later (after touching) greyish or pale ochraceous stipe, a whitish to pale brownish or greyish context in the stipe base, larger basidiospores, (7.5–)8.0–11×(5.0–)5.5–6.5(–7.0) µm (average = 9.3×5.9 µm), cheilocystidia of the excissa-type, and caulocystidia of three types (ANTONÍN *et al.* 2017). The phylogenetic positions of all the above-mentioned species also differ from that of *M. tristis*.

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