Identity of Agaricus brevipes Bull. (Melanoleuca brevipes, Tricholomataceae, Basidiomycota)

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ORIGINAL ARTICLE

Identity of *Agaricus brevipes* Bull. (*Melanoleuca brevipes*, *Tricholomataceae*, Basidiomycota)

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Abstract *Melanoleuca brevipes* (basionym *Agaricus brevipes*) represents one of the most frequently identified *Melanoleuca* species. However, there are several concepts of this taxon, and thus the identity of this species is unclear. This species was studied, based on the original table by Bulliard, macromorphological and micromorphological characters, and DNA sequences of recent collections and herbarium specimens. DNA sequences of ITS, RPB2 and tef1 markers revealed that specimens identified as *M. brevipes* belong to three species (*M. humilis, M. grammopodia,* and *M. malenconii*), where *M. humilis* or *M. malenconii* may resemble Bulliard's original species. According to these results, *Agaricus brevipes* is therefore considered a nomen dubium. A lectotype of *M. grammopodia* is proposed herein.

Keywords Taxonomy · Phylogeny · ITS · *M. humilis* · *M. grammopodia* · *M. malenconii*

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Introduction

Melanoleuca brevipes (Bull.) Pat., described by Bulliard (1791: tab. 521, 2; as *Agaricus brevipes*), is a traditionally recognised *Melanoleuca* species. It is included in all monographs of this genus, in keys and frequently also in lists of regional mycobiota (e.g., Bon 1990a; Boekhout 1999; Esteve-Raventós et al. 2007; Vesterholt 2012). It is usually characterised by robust, short-stiped basidiocarps, dark fuligineous, dark (greyish) brown to blackish pileus and stipe, dirty ochraceous, later fuligineous-ochraceous, yellowish grey or greyish brown lamellae, a brown context in the stipe base, and urticoid cystidia. During our work on the genus *Melanoleuca*, several specimens identified as *M. brevipes* were examined under a microscope and sequenced. However, all of them turned out to belong to *M. humilis* (Pers. : Fr.) Pat., *M. grammopodia* (Bull. : Fr.) Fayod, or *M. malenconii* Bon (= *M. sublanipes* Fontenla, Para & Vizzini). Also, the ITS sequence of a Swedish *M. brevipes* (JX429188) by Sánchez-García et al. (2013) was conspecific with *M. humilis*. In contrast, DNA sequences of *M. brevipes* (JF908351 and JF908352) identified by Vizzini et al. (2011) are closely related to the sequence of the holotype of *M. tristis* (personal data).

The aim of this study was to examine and sequence the ITS region of specimens agreeing macromorphologically and micromorphologically with *M. brevipes* and/or identified as *M. brevipes*, from various herbaria of various parts of Europe. Moreover, the type material of some selected *Melanoleuca* species was subjected to molecular analysis in order to elucidate their position within the genus. Additional sequences of translation elongation factor 1-alpha (tef1) and RNA polymerase–second largest subunit (RPB2) genes were determined to obtain further information on phylogenetic relationships between the specimens.

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Material and methods

Morphology

Macroscopic descriptions of collected specimens are based on fresh basidiocarps. Colour abbreviations follow Kornerup and Wanscher (1983), and herbarium abbreviations follow Thiers (2014). Authors of fungal names are cited according to the Authors of Fungal Names 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 (Tokyo, Japan) with a magnification of 1000×. 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). SEM microphotographs of basidiospores were taken using a Tescan Mira 3 LMU electron microscope.

Molecular methods

DNA was extracted from dried basidiocarps using standard kits (DNEasy Plant Mini Kit – Qiagen, PowerSoil DNA Isolation Kit – MoBio). In case of archival type herbarium specimens, a more complex protocol was used: the protocol of DNEasy Plant Minikit (Qiagen) was followed until step no. 11 (purification of DNA with AP2 buffer and centrifugation through QIAshredder Mini spin column) and then DNA extraction continued with the MagNA Pure Compact Nucleic Acid Isolation Kit I performed automatically in the MagNA Pure Compact Instrument (Roche). This automated magnetic bead technology enables a better yield of DNA and prevents contamination.

DNA fragments encompassing the ITS region of the nuclear ribosomal RNA gene were amplified using the primer combination ITS5/ITS4 Basidio (White et al. 1990; Nikolcheva and Bärlocher 2004). In case of older type specimens, the amplification occasionally gave ambiguous results. Therefore, genus-specific primers for the *Melanoleuca*-targeting ITS2 region were developed using Genefisher2 software (http://bibiserv.techfak.uni-bielefeld.de/genefisher2/submission.html): MELITS2F (5′-TTGGCTCTCGCATCGA-3′) and MELITS2R (5′-CTCGGACAGCCAGAGA-3′). The amplified DNA fragment was approximately 470 bp long, encompassing partial 5.8S, complete ITS2, and partial LSU regions. The ITS region was amplified with PCR as described earlier (Tomšovský 2012); for MELITS2F /MELITS2R primers, the annealing temperature was optimised to 52 °C and the number of cycles was set to 38.

The tef1 gene was amplified according to Antonín et al. (2013, 2014). The 6–7 domain region of RPB2 gene was amplified using the primer combination fRPB2-5F/bRPB2-7.1R and the PCR regime according to Matheny (2005).

All PCR reactions were performed using the Mastercycler_ep thermocycler (Eppendorf, Hamburg, Germany). Amplicons were custom-purified and sequenced at Macrogen, Inc. (Seoul, Republic of Korea). The obtained sequences were analysed and manually adjusted in Chromas Lite 2.1.1. (Technelysium, Australia). The sequences were deposited in the NCBI Nucleotide Sequence Database, and their accession numbers are given in Table 1.

Phylogenetic analysis

The data set of ITS sequences was completed with *Melanoleuca* sequences previously published by Vizzini et al. (2011), Sánchez-García et al. (2013), and Antonín et al. (2014), aligned in Mafft v. 7, using Q-INS-i strategy (Katoh and Standley 2013). The data set included a total of 771 positions, of which 492 were conserved, 226 were variable and 86 were singleton sites as determined in MEGA 6.06 (Tamura et al. 2013).

A combined data set of RPB2, ITS and tef1 was created. The partition homogeneity test implemented in Paup (Swofford 2003) confirmed the homogeneity of the phylogenetic signal of individual markers (p value=1.00). The data set was completed with outgroup *M. verrucipes*, AFTOL−ID 818 (sequences no. DQ474119, DQ490642, GU187726). The data set included a total of 2,278 positions, of which 1,837 were conserved, 418 were variable and 236 were singleton sites as determined in MEGA 6.06 (Tamura et al. 2013).

Bayesian analyses were run in MrBayes 3.2.3 (Huelsenbeck and Ronquist 2003). Likelihood settings from the best-fit model (TPM2uf + G = ITS dataset; TrNef + G = combined data set) were selected by the Bayesian information criterion in jModelTest2 (Darriba et al. 2012). We ran four chains for 10 million generations. The burn-in value (1 million generations) was estimated in Tracer v. 1.5 (Rambaut and Drummond 2009). Sampling frequency was set to every 100th generation. The data from the Bayesian analyses were deposited in the Treebase (ID 17947). The additional phylogenetic analyses of both data sets were carried out in PHYML, estimating maximum-likelihood phylogenies and running at the server Phylogeny.fr (Dereeper et al. 2008) using the "A la Carte" mode. Bootstrap branch support values (BP) were estimated in PHYML under the maximum-likelihood criterion using the default of 100 replicates.

Results

Phylogeny

The 50 % majority-rule consensus tree from the Bayesian analysis of both data sets (Figs. 1 and 2) confirmed our previous hypothesis on the genetic variety of "*M. brevipes*-like" specimens. The maximum-likelihood analyses resulted in

Table 1 The newly sequenced specimens in the study. The specimens with a short stipe (stipe length ≤ pileus width) are marked in bold

Species	Locality	Herbarium specimen no.	Genbank Acc. No. (ITS, tef1, and RPB2)
M. decembris, holotype	France, Vers Col de la Croix de Fer	LIP 85110	KP192292
M. grammopodia	Czech Rep., Roudnice nad Labem, Dušníky	BRNM 761817	KP192266
M. grammopodia	Czech Rep., Rašeliniště Kačerov National Nature Reserve	BRNM 710012	KP192265
M. grammopodia	**Czech Rep., Brno, Lesná**	**BRNM 762049**	**KP192270**
M. grammopodia, epitype	Czech Rep., Podyjí National Park, Mašovice	BRNM 761800	KP192272
M. grammopodia	**Czech Rep., Třemošnice**	**BRNM 762047**	**KT279047**
			KT279048
			KT279059
M. grammopodia	**Denmark, Copenhagen, Østerbro**	**SLO 1461**	**KP192271**
M. grammopodia	**Slovakia, Veľká Fatra Mts., Vrchlúky**	**SLO 1463**	**KP192264**
			KT279049
			KT279058
M. grammopodia	**Slovakia, Liptovské Revúce**	**SLO 1468**	**KP192267**
			KT279051
			KT279061
M. grammopodia	**Slovakia, Liptovské Revúce**	**SLO 1466**	**KP192269**
			KT279050
			KT279060
M. grammopodia	**Slovakia, Bratislava, Rača**	**SLO 1465**	**KP192281**
M. grammopodia	Slovakia, Muránska Zdychava	SLO 1469	KP192268
M. grammopodia f. *macrocarpa*, holotype	Netherlands, Leusden	L 0649917	KP192293
M. grammopodia var. *obscura*, holotype	France, St. Valery	LIP 741017	KP192284
M. humilis	Czech Republic, Přerov	BRNM 751965	KJ425530
			KJ425543
			KT279057
M. humilis	**Czech Republic, Kroměříž**	**BRNM 710023**	**KJ425531**
			KJ425544
			KT279055
M. humilis	**Czech Rep., České Budějovice**	**CB**	**KP192290**
			KT279052
			KT279056
M. humilis	**Norway, Oslo, Tøyenhagen**	**W 10087**	**KP192287**
M. humilis	**France, Maule, Parc d'Aulnay,**	**LIP, RC/F00.001**	**KP192289**
M. humilis	**Slovakia, Slanická osada**	**SLO 1459**	**KP192274**
M. humilis	Slovakia, Námestovo	SLO 1460	KP192273
M. malenconii	Czech Rep., Roudnice nad Labem, Kleneč	BRNM 762051	KP192275
			KT279053
			KT279062
M. malenconii	Czech Rep., Brno	BRNM 762046	KP192276
M. malenconii, holotype	France, Lieu La Grande Motte	LIP, M. Bon 3474	KP192294
M. malenconii	**France, Wingles**	**LIP, RC/F90.058**	**KP192295**
M. malenconii	**Slovakia, Slanícka osada**	**SLO 1453**	**KP192279**
M. malenconii	**Slovakia, Bratislava**	**SLO 1455**	**KP192277**
			KT279054
			KT279063
M. malenconii	**Slovakia, Bratislava**	**SLO 1456**	**KP192278**
M. media	France, Ecault	LIP 84320	KP192282
M. metrodii, holotype	France, Abbeville	LIP 72092943	KP192291

Table 1 (continued)

Species	Locality	Herbarium specimen no.	Genbank Acc. No. (ITS, tef1, and RPB2)
M. politoinaequalipes, holotype	France, Béguet, SaintVincent des Landes	LIP 650403	KP192283
M. pseudobrevipes, holotype	France, Wimereux	LIP 71120235	KP192288
M. pseudoevenosa, holotype	France, Bergons, Haute Pyrenées	LIP 771108	KP192285
M. subbrevipes	**France, Vendee, Le Fenouiller**	**LIP 02129**	**KP192286**
M. tristis, holotype	Serbia, Tara, Kaludjarskoe Bare	IB 1963/0722	KP192280

phylogenetic tree topologies almost identical to the Bayesian ones. Specimens having the morphology of *M. brevipes* mostly fell in *M. grammopodia, M. humilis* and to a lesser extent in *M. sublanipes*. Therefore, we treat *M. brevipes* as a dubious name. *Melanoleuca sublanipes* turned out to be conspecific with *M. malenconii* Bon (Bon 1990c), which is the older, correct name. Also sequences of some other type specimens revealed their identity. Some of them were conspecific with *M. grammopodia* (*Melanoleuca grammopodia* var. *obscura* Bon, *M. pseudobrevipes* Bon, *M. pseudoevenosa* Bon ex Bon & G. Moreno, *M. politoinaequalipes* Beguet ex M. Traverso & Zotti) or *M. humilis* (*M. decembris* Métrod ex Bon, *M. grammopodia* f. *macrocarpa* Boekhout, *M. metrodii* Bon).

Taxonomy

Melanoleuca humilis (Pers.) Pat., Catal Pl Cellul Tunisie: 22, 1897. (Figs. 3b, 4b, and 5)

≡ *Agaricus humilis* Pers. : Fr., Syst Mycol I: 51, 1821. – *Tricholoma humile* (Pers. : Fr.) Quél., Mém Soc Emul Montbél II, 5: 317, 1872. – *Gyrophila humilis* (Pers. : Fr.)

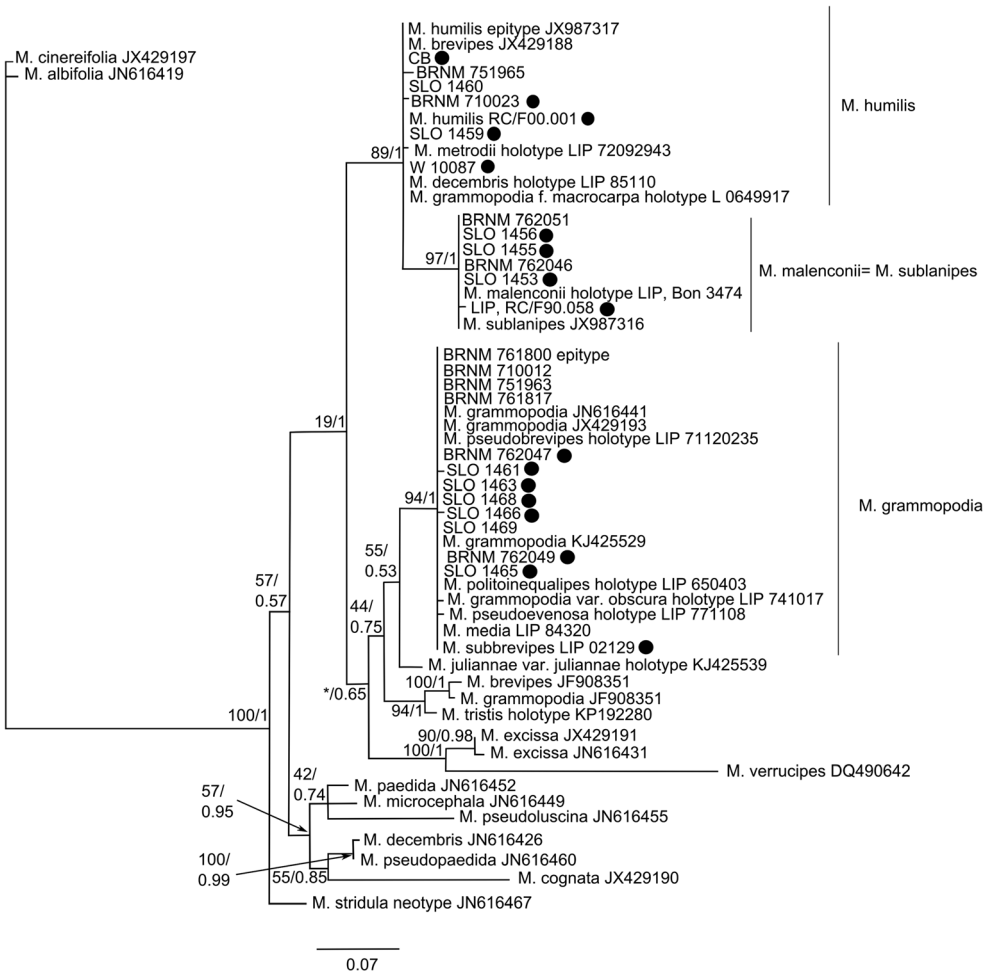

Fig. 1 Phylogenetic tree of the ITS region based on Bayesian analysis (for legends to the numbers, see Table 1). The specimens with a short stipe (stipe length ≤ pileus width) are marked with *black circles*. Numbers at branches indicate Maximum-likelihood bootstrap proportion and Bayesian posterior probability values. The asterisk (*) marks a different topology in both analyses. The bar indicates the number of expected substitutions per position

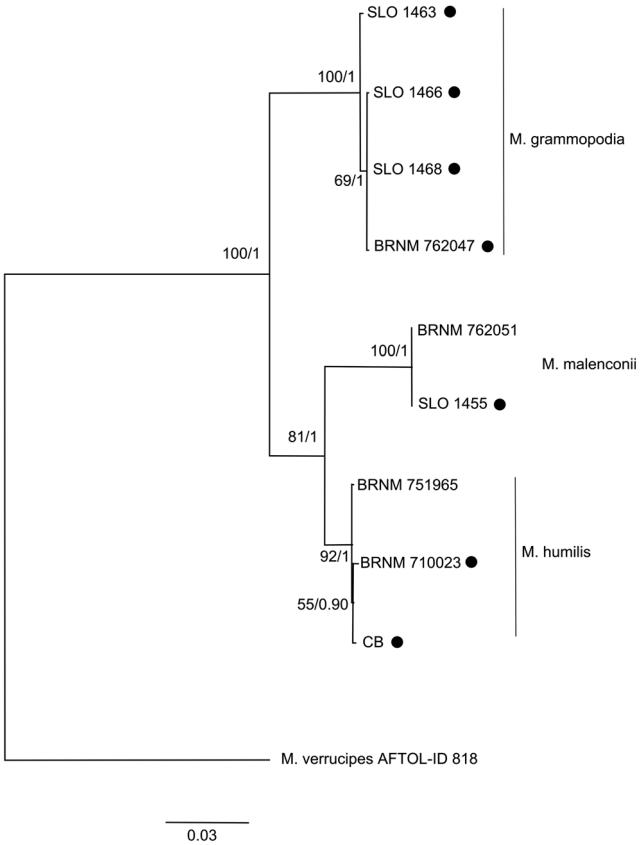

Fig. 2 Phylogenetic tree of combined RPB2-ITS-tef1 data set based on Bayesian analysis (for legends to numbers, see Table 1). The specimens with a short stipe (stipe length ≤ pileus width) are marked with *black circles*. Numbers at branches indicate Maximum-likelihood bootstrap proportion and Bayesian posterior probability values. The bar indicates the number of expected substitutions per position

Quél., Enchir Fung: 18, 1886. – *Tricholoma melaleucum* var. *humile* (Pers. : Fr.) L. Maire, Étude Synth Genre *Tricholoma*: 34, 1916.

= *Melanoleuca metrodii* Bon, Doc Mycol 12(46): 32, 1982. (nom. nud.; without a Latin diagnosis and a typus indication).

= *Melanoleuca decembris* Métrod ex Bon, Bull Féder Mycol Dauphiné-Savoie 102: 22, 1986.

= *Melanoleuca grammopodia* f. *macrocarpa* Boekhout, Persoonia 13(4): 407, 1988.

Lectotype Buxbaum, Plantarum minus cognotarum, cent. IV, tab. 31, 1733 (indicated by Fries 1821).

Epitype Italy, Monte Nerone di Piobbico (PU), 25 Aug. 2003 leg. R. Paolini (ANC M0225) (Vizzini et al. 2012).

Pileus up to 70 mm broad, convex, then applanate to (slightly) funnel-shaped, slightly umbilicate and with a low broad umbo, inflexed to involute at margin, undulate when old, smooth, glabrous, leathery, grey-brown (5D2, 6D3–4), paler towards margin. Lamellae crowded, L = c. 70–80, l= 3–4, emarginate and attached with a tooth, greyish or beige, with concolorous, finely pubescent edge. Stipe 22–65×5–10 mm, cylindrical, with clavate, up to 15 mm wide base, pruinose or finely floccose especially at apex, finely longitudinally fibrillose (more distinctly when drying out), dark watery brown when moist, greyish (6C2–3) when dried out. Context whitish in pileus, greyish brownish under pileipellis, brownish or rusty brown in stipe centre and (dark) brown in stipe base, with slight fungoid smell and mild, after some time slightly astringent taste.

Basidiospores 7.0–10×(4.5)5.0–6.5(7.0) μm, average= 8.6×5.6 μm, E=1.31–1.81, Q=1.43–1.68, (broadly) ellipsoid, ellipsoid-fusoid, obovoid, verruculose, warts round or irregular, variable in size, mixed with scattered ridges, amyloid. Basidia 28–45×8.0–13 μm, 4-spored, clavate. Basidioles 13–40×5.0–13 μm, clavate, subcylindrical, subfusoid. Cheilocystidia 27–60×5.0–11 μm, rostrum 3.5–4.5 μm wide, urticoid of the brevipes-, less frequently exscissa-type, basal part clavate, subfusoid, vesiculose, often irregular, thin-walled, with or without apical crystals. Marginal cells 15–32×6.0–11 μm, variable in shape, clavate, fusoid, sublageniform, regular to irregular, thin-walled. Pleurocystidia not observed. Trama hyphae cylindrical to inflated, thin-walled, non-dextrinoid, up to 15 μm wide. Pileipellis an ixocutis, composed of cylindrical, radially arranged, thin-walled, non-dextrinoid 3.0–9.0(11) μm wide hyphae; terminal cells 31–66×6.0–10(14) μm, appressed to erect, clavate, fusoid, cylindrical, thin-walled, regular to irregular, rarely scatteredly diverticulate, with greyish-brownish pigmentation. Stipitipellis a cutis of cylindrical, parallel, smooth, thin-walled to slightly thick-walled, non-dextrinoid, 3.0–6.0 μm wide hyphae. Caulocystidia single or in groups, of two types: (1) (17)22–65×7.0–12 μm, clavate, cylindrical, subfusoid, thin-walled, ± regular cells, and (2) scattered, 46–55×8.0–9.0 μm, urticoid, subulate or fusoid, thin-walled cells. Clamp connections absent.

Specimens examined

Czech Republic: České Budějovice, Stromovka forest park, meadow, 23 Apr 2010 leg. M. Vašutová, det. M. Beran (CB, as *M.* cf. *brevipes*). – Kroměříž, Podzámecká zahrada garden, ruderalised place, under *Picea abies*, *Carpinus* and *Ulmus*, 19 Apr 2008 leg. V. Antonín (08.01) and S. Komínková (BRNM 710023). – Přerov, sludge reservoir, on sandy soil, 8 Apr 2007 leg. J. Polčák (BRNM 751965). – Přerov, artificial grass stand, under *Tilia*, 7 Apr 2010 leg. J. Polčák (BRNM 762083). France: Vers Col de la Croix de Fer, grassy path in calcareous fir forest, alt. 1700 m, 6 Sep 1985 leg. et det. M. Bon (holotype of *M. decembris*, LIP 85110). – Abbeville, garden, stony path, 29 Sep 1972 leg. Mrs. Bon, det. M. Bon (holotype of *M. metrodii*, LIP 72092943). – Maule, Parc d'Aulnay, calcareous road margin under *Fraxinus*, *Alnus* and *Quercus*, 12 Mar 2000 leg. G. Redeuilh, det. R. Courtecuisse (LIP, RC/F00.001, as *M. brevipes*). Netherlands: prov. Utrecht, Leusden, 2 Nov 1977 leg. Erkelens (holotype of *M. grammopodia* f.

macrocarpa, L 0649917). Norway: Oslo, Tøyenhagen, in grass, 25 Sep 1973 leg. et det. A.-E. Torkelsen (W 10087, as *M. brevipes*). Slovakia: Západné Beskydy Mts., Slanická osada, road-side, in grass, under *Picea* and *Thuja*, 11 Oct 2012, leg. N. Rybáriková (SLO 1459). – Západné Beskydy Mts., Námestovo, ruderalised place, on soil under *Picea*, 11 Oct 2012, leg. S. Adamčík (SLO 1460).

Remarks: *Melanoleuca humilis* is characterised by having an up to 70 mm broad, grey-brown pileus, greyish or beige lamellae, a dark watery brown stipe pallescent to greyish, a brown coloured stipe base context, (7.5)8.0–10×5.0–6.5 μm, usually (broadly) ellipsoid, ellipsoid-fusoid or obovoid, verruculose basidiospores, urticoid cheilocystidia of the brevipes-, less frequently exscissa-type, and caulocystidia of two types.

Melanoleuca grammopodia (Bull. : Fr.) Pat., Catal Pl. Cellul Tunisie: 22, 1897. (Figs. 3a, 4a, 6, 7, 9a)

≡ *Agaricus grammopodius* Bull., Herb France 12: t. 548, 1792. – *Tricholoma grammopodium* (Bull.) Quél., Mém Soc Émul Montbéliard 5: 83, 1872. – *Gyrophila grammopodia* (Bull.) Quél., Enchir Fung: 17, 1886. – *Melaleuca grammopodia* (Bull.) Fayod, Ann Sci Natur Bot 9: 348, 1889. – *Tricholoma melaleucum* var. *grammopodium* (Bull.) Maire, Bull Soc Hist Natur Afrique Nord 7: 28, 1916.

= *Melanoleuca grammopodia* var. *obscura* Bon, Doc Mycol 20(79): 60, 1990.

= *Melanoleuca pseudobrevipes* Bon, Doc Mycol 20(79): 59, 1990.

= *Melanoleuca pseudoevenosa* Bon ex Bon & G. Moreno, Doc Mycol 11(43): 37, 1980.

= *Melanoleuca politoinaequalipes* Beguet ex M. Traverso & Zotti, Bull Sem Feder Assoc Mycol Méditerr n.s. 22: 32, 2002.

Lectotype Bulliard, Herbier de la France: tab. 548, 1791 (as *Agaricus grammopodius*).

Epitype Podyjí National Park, Mašovice, Mločí údolí valley, alluvium, under *Carpinus, Fraxinus* and *Alnus glutinosa*, alt. 300–320 m, 31 Aug 2012 leg. V. Antonín 12.158 (BRNM 761800, designated here).

Pileus 54–170(260) mm broad, low convex or low conical, then applanate, depressed at centre with a (broad) low obtuse umbo, involute at margin or not, not hygrophanous, smooth, glabrous, apparently leathery, sometimes cracking at margin, grey-brown (6D4, 6F5, 7E–F4–6), especially at centre, often paler, greyish, whitish or with brownish tinge only (e.g., 4–5B3) towards margin. Lamellae close, L = c. 60–100, l=3–5, emarginate and attached to slightly decurrent with tooth, sometimes furcate near the stipe, rather narrow, usually ± horizontal, cream-coloured or greyish beige (±6C2), sometimes becoming slightly brownish after touching, with concolorous, finely pubescent edge. Stipe 17–110×7–15 mm, cylindrical, slightly broadened at apex, clavate-bulbose (11–25 mm) at base, finely fibrillose-floccose at apex, (distinctly) fibrillose otherwise, fibrils pale (grey-)brown (±6C–D3–5, 7D3); basal tomentum white. Context whitish, greyish-brownish under pileipellis, woolly-fibrillose and dirty whitish or greyish in stipe, with spermatic, pleasantly farinaceous or musty-farinaceous smell.

Basidiospores (6.0)7.0–10(11.5)×(4.0)4.5–6.0(7.0) μm, average=9.4×5.5 μm, E=1.25–2.02, Q=1.55–1.83, ellipsoid, fusoid-ellipsoid, verruculose with scattered connections, amyloid. Basidia (25)32–48×9.0–14.5 μm, 4-spored, clavate, rarely subfusoid or subcapitate. Basidioles 15–45(53)×4.5–14 μm, clavate, subcylindrical. Cheilocystidia urticoid of the exscissa-type, rarely brevipes-type, (16)25–57×(3.0)5.0–10(13) μm, with fusoid, subglobose, clavate, sometimes slightly irregular base and subulate or subcylindrical, 2.0–4.0 μm wide apex, thin-walled, with or without apical crystals. Marginal cells 14–35(42)×4.0–8.0(10) μm, clavate, cylindrical, fusoid, regular, irregular to branched, thin-walled. Pleurocystidia not observed. Trama hyphae cylindrical to subinflated, thin-walled, non-dextrinoid, up to 15 μm wide. Pileipellis an ixocutis, transient to subtrichoderm at centre, composed of cylindrical, ± radially arranged, thin-walled to slightly thick-walled, non-dextrinoid, 3.0–8.0(10) μm wide hyphae; terminal cells appressed to erect, cylindrical to (narrowly) clavate, subfusoid, thin-walled, up to 8.0 μm wide, with vacuolar-incrusting pigmentation, yellowish or ochraceous grey in KOH. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth or minutely incrusted, non-dextrinoid, 2.5–7.0 μm wide hyphae with greyish walls in KOH. Caulocystidia of two types: (1) (15)24–62(98)×3.5–11 μm, appressed to erect, clavate, cylindrical, subfusoid, obtuse, thin-walled to slightly thick-walled, sometimes septate

Fig. 3 Colour of context. **a**. *Melanoleuca grammopodia* (SLO 1463), **b**. *M. humilis* (SLO 1460), **c**. *M. malenconii* (SLO 1456). Photos O. Ďuriška

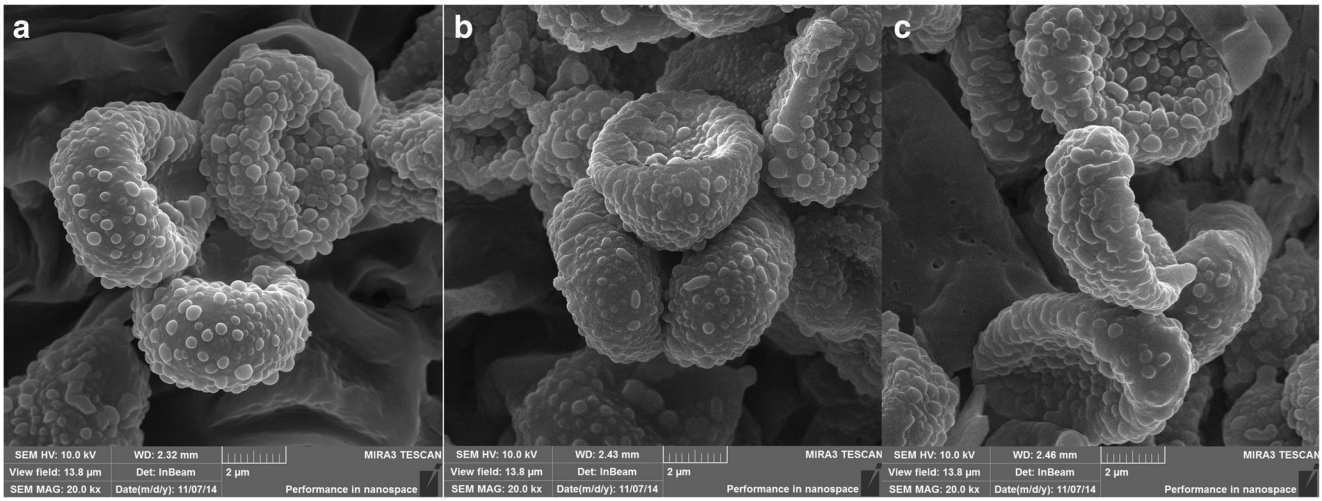

Fig. 4 SEM microphotographs of basidiospores. **a**. *M. grammopodia* (BRNM 710012), **b**. *M. humilis* (BRNM 762083), **c**. *M. malenconii* (LIP 3474). Photos L. Ilkovics

cells, and (2) scarce 25–45×4.0–9.0 µm, thin-walled, urticoid cells of the exscissa-type, with fusoid base. Basal mycelium of cylindrical, ± thin-walled, up to 6.0 µm wide hyphae with subulate lateral projections. Clamp connections absent.

Specimens examined

Czech Republic: Dušníky near Roudnice nad Labem, 9 Nov 2008 leg. D. Marounek (BRNM 761817). – Brandýs nad Labem, Vaňkovo jezero lake, in sparse grass on sandy soil, 6 Nov 2012 leg. J. Borovička (BRNM 761819). – Rataje nad Sázavou, stream valley under Mirošovský kvartýr, road-side under *Picea* and broadleaved trees, 12 Aug 2010 leg. J. Borovička (BRNM 761818). – Třemošnice near Čáslav, village street, under *Populus nigra* in a sparse grass stand, 26 Oct 2008 leg. J. Borovička (BRNM 762047). – Orlické hory Mts., Kačerov, Rašeliniště Kačerov National Nature Reserve, on soil in grassy stand along road, alt. 660–700 m, 25 Sep 2007 leg. M. Beran (Antonín 07.288, BRNM 710012). – Orlické hory Mts., Pěčín, pars Přím, conjunction of the Zdobnice and Říčka rivers, in grass by forest path in spruce-beech forest, alt. 450–500 m, 26 Sep 2007 leg. J. Burel and J. Zavřel (Antonín 07.298, BRNM). – Mohelno, Mohelno Serpentine Steppe National Nature Reserve, alluvium, under *Quercus, Fraxinus* and *Euonymus*, alt. 270 m, 19 Sep 2013 leg. V. Antonín (13.261) and H. Ševčíková (BRNM 761798). – Brno, Brněnská přehrada dam, part named Obora, ruderalised place (*Datura, Urtica*) in spruce forest clearing, 24 Nov 2013 leg. R. Schles (BRNM 761799). – Brno-Lesná, in the direction of Soběšice, on soil under *Pinus* and *Quercus*, 6 Nov 2012 leg. A. Vágner (BRNM 762049). – Ždánický les hills, Bučovice-Kloboučky, Radlovec valley, road-side in *Fraxinus*, *Acer pseudoplatanus* and *Larix* stand on alluvium, alt. c. 250 m, 18 Oct 2008 leg. V. Antonín 08.304 (BRNM 762048). – Bílé Karpaty Mts., Slavkov u Uherského Brodu, Porážky National Nature Reserve, grassland, 9 Oct 2007 leg. I. Jongepierová and K. Devánová (BRNM 751964). – Podyjí National Park, Mašovice, Mločí údolí valley, alluvium, under *Carpinus, Fraxinus* and *Alnus glutinosa*, alt. 300–320 m, 31 Aug 2012 leg. V. Antonín 12.158 (epitype, BRNM 761800).

Fig. 5 *Melanoleuca humilis*. Basidiocarps (BRNM 710023). Photo V. Antonín

Fig. 6 *Melanoleuca grammopodia*. Basidiocarps of a long-stiped form (epitype, BRNM 761800). Photo V. Antonín

Fig. 7 *Melanoleuca grammopodia*. Basidiocarps of a short-stiped form (SLO 1463). Photo O. Ďuriška

Denmark: Copenhagen, Østerbro, on rich soil in park, 9 Oct 2012, leg. Thomas Læssøe (SLO 1461). – Bullerup, in mossy, unfertilised grass lawn on sandy soil, 22 Sep 2011, leg. Leif Wegge Laursen (SLO 1462). – France: Pas-de-Calais, Saint-Etienne-au-Mont, dunes d'Ecault, sandy grassland, 3 Nov 1984 leg. et det. M. Bon 84320 (LIP, as *M. media*). – Bergons, Hautes-Pyrénées, grassland with *Calluna*, 8 Nov 1977 leg. et det. M. Bon (holotype of *M. pseudoevenosa*, LIP 771108). – Pas-de-Calais, Wimereux, cut grassland (*Cynosurion*), 2 Dec 1971 leg. et det. M. Bon (holotype of *M. pseudobrevipes*, LIP 71120235). – Pas-de-Calais, St-Valery-sur-Somme, mossy grassland, 17 Oct 1974 leg. et det. M. Bon (holotype of *M. grammopodia* var. *obscura*, LIP 741017). – Loire-Atlantique Dpt., Béguet, Saint-Vincent-des-Landes, grassy broadleaved forest, Apr 1965 leg. A. Bequet (holotype of *M. politoinaequalipes*, LIP 650403). – Vendée, Le Fenouiller, prairie, Feb 2011 leg. R. Pacaud, det. M. Bon (LIP 02129, as *M. subbrevipes*). Slovakia: Zvolenská kotlina, Zvolen, Borová hora Arboretum, grassland, alt. 300–360 m, 28 Sep 2009 leg. I. Kautmanová and V. Antonín 09.291 (BRNM 751963). – Zvolenská kotlina, Zvolen, Borová hora Arboretum, alt. 300 m, under *Tilia*, *Quercus robur*, *Carpinus betulus* and *Acer campestre*, 5 Nov 2009, leg. S. Glejdura (SLO 1470). – Malé Karpaty Mts., Bratislava – Rača, Biely kríž, broadleaved forest, road side, soil with sawdust, alt. 500 m, 7 Sep 2013, leg. O. Ďuriška (SLO 1465). – Veľká Fatra Mts., Vrchlúky, soil under *Corylus avellana*, 10 Sep 2013, leg. S. Jančovičová (SLO 1463). – Veľká Fatra Mts., Liptovské revúce, Suchá dolina, pasture, in grass, 29 Sep 2013, leg. O. Ďuriška (SLO 1464). – Stolické vrchy Mts., Muránska Zdychava, road side, margin of meadow, alt. 660 m, under *Alnus glutinosa* and *Tilia cordata*, leg. S. Glejdura (SLO 1469). – Veľká Fatra Mts., Liptovské Revúce, near shepherd's hut, pasture, in grass, 29 Sep 2013, leg. O. Ďuriška (SLO 1466). – Veľká Fatra Mts., Ružomberok, Suchá hôrka, on soil, under *Corylus avellana* and *Crataegus* sp., alt. 623 m, 13 Sep 2013, leg. O. Ďuriška (SLO 1467). – Veľká Fatra Mts., Liptovské Revúce, Suchá dolina, pasture, in grass, 29 Sep 2013, leg. O. Ďuriška (SLO 1468).

Remarks: *Melanoleuca grammopodia* is characterised by having a 70–125 mm broad, centrally grey-brown and marginally greyish, whitish or brownish tinged pileus, cream or greyish beige lamellae, a rather robust, long or short (long-stiped and short-stiped forms), (grey-) brown stipe, a whitish stipe base context, 8.5–10(−11)×4.75–6.0(−6.5) µm, ellipsoid, fusoid-ellipsoid, verruculose basidiospores, urticoid cheilocystidia of the exscissa-, rarely brevipes-type, and caulocystidia of two types.

Melanoleuca malenconii Bon, Doc Mycol 20(79): 59, 1990. (Figs. 3c, 4c, and 8)

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

Holotype France, Lieu La Grande Motte (34), *Pinus pinea*, on sand, 15 November 1985 leg. Chevassut, det. M. Bon 3474 (LIP, as *M. turrita*).

Pileus 20–70 mm broad, slightly convex with reflexed margin and slightly umbonate centre when young, then applanate or centrally slightly depressed, with ± straight margin and central broad, sometimes less distinct umbo, smooth, slightly pubescent (especially at margin) when young, later (soon?) ± glabrescent, in some collections apparently brown-black tomentose at centre, hygrophanous or not, not translucently striate, grey, ochraceous to dirty yellow (4–5B3, 5–6B4, 6C1–2, 7D2), uniformly coloured or with dark brown (7E3–4, 7E–F8) centre, sometimes with watery stains around centre when old. Lamellae moderately close, L=50–60, l=2–4(5), emarginate and adnate with tooth, white to cream, then up to pale to greyish yellow (up to 4A–B3), with beige reflex, edge concolorous, finely pubescent. Stipe 22–50×3–6(7) mm, cylindrical or slightly laterally compressed, slightly broadened at apex, ± clavate to bulbose (up to 18 mm broad) at base, longitudinally fibrillose, slightly striate (lamellae) at apex when

Fig. 8 *Melanoleuca malenconii*. Basidiocarps (SLO 1453). Photo O. Ďuriška

young, slightly pruinose-pubescent to distinctly floccose, rarely subglabrous at apex, sometimes especially in lower part floccose-hairy, grey-brown (6E–F3–4, 7D3, 7E4), uniformly when old, with paler apex when young; with or without white basal tomentum. Context whitish to pale brownish in pileus, dirty grey-brown, dark brown or rusty brown in stipe base, without distinct smell or with slightly fungoid smell and with mild or bitterish taste.

Basidiospores (6.0)7.4–10×(4.5)5.0–6.0(7.0) μm, average=8.8×5.7 μm, 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×(8)9.0–13 μm, 4-spored, clavate. Basidioles 12–40×5.0–12 μm, clavate, subcylindrical. Cheilocystidia urticoid, of both the brevipes-type and exscissa-type, 22–60×3.0–14 μm, rostrum (1.5)2.75–5.0 μm wide, basal part fusoid to subcylindrical, thin-walled, apex subulate or cylindrical, obtuse, thin-walled to slightly thick-walled, with or without apical crystals. Marginal cells 26–32×5.5–10 μm, clavate, fusoid, irregular or with projection, thin-walled. Pleurocystidia similar to cheilocystidia, sometimes absent. Pileipellis an ixocutis to an ixotrichoderm (margin), almost subixotrichoderm at centre, composed of ± cylindrical, ± thin-walled, non-dextrinoid, up to 8.0(10) μm wide hyphae; terminal cells up to 60×5.0–10 μm, appressed to erect, cylindrical, narrowly clavate, subfusoid, obtuse. Stipitipellis a cutis of cylindrical, parallel, ± thin-walled, non-dextrinoid, up to 6.0(8.0) μm wide hyphae. Caulohymenium of three types: (1) 16–50×3.0–15 μm, clavate or cylindrical, thin-walled cells, (2) 2-spored (always?), clavate basidia, and (3) urticoid cystidia, of the excissa-type or ± brevipes-type, similar to cheilocystidia, 24–60×5.5–9.0(12) μm, rostrum 3.0–5.0 μm; cystidia and caulobasidia sometimes very rare (or even absent?). Clamp connections absent.

Specimens examined

Czech Republic: Roudnice nad Labem, Kleneč, artificial grassy stand, 6 Dec 2008 leg. J. Borovička, M. Kříž and D. Marounek (BRNM 762051). – Brno, courtyard of the Moravian Museum, artificial lawn, 5 Nov 2009 leg. V. Antonín 09.357 (BRNM 762046). France: Lieu La Grande Motte (34), *Pinus pinea*, on sand, 15 Nov 1985 leg. Chevassut, det. M. Bon 3474 (LIP, as *M. turrita*, holotype of *M. malenconii*). – Wingles, Pas-de-Calais, base of heap of Loira slate under young *Betula*, 17 Oct 1990 leg. et det. R. Courtecuisse (LIP, RC/F90.058, as *M. brevipes*). Italy: Veneto, Padova, Montegrotto Terme, hotel garden, 7 Nov 2008 leg. G. Zecchin (ANC M0222, holotype of *M. sublanipes*). – Emilia-Romagna Prov., Ravenna, Lido di Dante, sand dunes, in grass under *Pinus maritima* and *Quercus ilex*, 9 Nov 2000 leg. V. Antonín 00.229 (BRNM 762049). – Emilia-Romagna Prov., Ravenna, Marina di Ravenna, on soil in private garden, 5 Nov 2007 leg. V. Antonín 07.419 and M. Marcheggiani (BRNM 761883). Slovakia: Podunajská nížina, Bratislava, Apollo business centre, in lawn, 24 Oct 2010, leg. V. Zárecká (SLO 1451). – Podunajská nížina, Bratislava, Petržalka, in garden, 30 Oct 2012, leg. Čačaná (SLO 1452). – Západné Beskydy, Slanícka osada, in grass and *Equisetum*, under *Thuja*, 11 Oct 2012, leg. O. Ďuriška (SLO 1453). – Podunajská nížina, Bratislava, Botanical garden, in lawn, under *Thuja*, 16 Nov 2012, leg. O. Ďuriška (SLO 1455). – Malé Karpaty, Bratislava, in grass in aviary, 8 Sep 2012, leg. D. Ďuriška (SLO 1456). – Podunajská nížina, Bratislava, Botanical garden, in lawn, along path, under *Larix gmelini*, 16 Nov 2012, leg. O. Ďuriška (SLO 1457). – Podunajská nížina, Bratislava, Airport, mulch bark and needles, under *Picea*, 3 Oct 2012, L. Pomšár (SLO 1458). – Malé Karpaty, Bratislava, Krasňany, in grass, under *Pinus* sp., 12 Nov 2012, leg. I. Kautmanová (SLO 1454).

Remarks: *Melanoleuca malenconii* is characterised by having a 20–70 mm broad, slightly pubescent (especially at margin), later ± glabrescent, grey, ochraceous to dirty yellow pileus, white to cream, then up to pale to greyish yellow lamellae, a rather uniformly grey-brown stipe slightly pruinose-pubescent to distinctly floccose at apex, sometimes (especially in lower part) floccose-hairy, a brown to grey-brown context in the stipe base, (7.0)8.0–10×5.0–6.0(6.5) μm, (broadly) ellipsoid or ovoid basidiospores, urticoid cheilocystidia, often developed pleurocystidia similar to cheilocystidia, a pileipellis in the form of an ixocutis to an ixotrichoderm, and presence of a caulohymenium.

Vizzini et al. (2011) published a new species named *M. sublanipes* Fontenla et al., and one year later, Vizzini et al. (2012) published an additional description and note to this species and the closely related *M. humilis*. The same authors (Fontenla et al. 2003) synonymised *M. malenconii* with *M. turrita* s. Malençon (Malençon and Bertault 1975). However, our phylogenetic studies prove that *M. sublanipes* is identical to the older species name *M. malenconii* Bon (Bon 1990c).

Discussion

According to the original description and figure *Melanoleuca brevipes* (Bull.: Fr.) Pat. is characterised by having robust fleshy basidiocarps with a grey or fuligineous grey and centrally brownish pileus, a thick, short (only 20–25×5–10 mm large) and glabrous stipe, greyish lamellae, and a ferrugineous stipe context (lectotype: Bulliard, Herbier de la France 11, tab. 521, 2, 1791, as *Agaricus brevipes*). However, the species concept differs among various authors. Gröger (2006) treats it as a separate, well-defined species characterised by a moderately long to short stipe, whitish cream, grey cream to grey

brownish coloured lamellae, a dark brown stipe base context, ellipsoid, finely to moderately warty spores, urticoid cystidia, and by growing in spring and autumn. Bon (1990a) also considered it an independent species with an almost concolorous, dark fuligineous to dark brown or blackish pileus and stipe, dirty ochraceous, fuligineous-ochraceous to grey yellowish lamellae, a brown context in the stipe base, and also urticoid cystidia (Bon 1990a). In contrast, *M. brevipes* in the sense of Arnolds and J.E. Lange represents a macrocystidiate taxon, *M. langei* (Boekhout) Bon (= *M. polioleuca* f. *langei*); Bon (1990a) synonymised *M. brevipes* in the sense of Ricken with *M. contracta* Métrod. Bresadola (1928) described and depicted *M. brevipes* with macrocystidia. According to Gröger (2006), it represents *M. grammopodia*, while Singer (1943) invalidly described Bresadola's fungus as *M. brevipes* f. *bresadolae* Singer, and Bon (1978) invalidly raised it to species rank. However, according to Fontenla and Para (2013), all specimens identified as *M. brevipes* by Bresadola and preserved in Bresadola's herbarium typically have urticoid cheilocystidia. The concept of *M. brevipes* by Singer (1935), Bon (1990a) and probably also Watling and Turnbull (1998) is mainly based on the concept by Kühner (1978), who adopted the concept from Konrad and Maublanc (1924–1933). It seems to be a concept closest to Bresadola's original table. The short description by Quélet (1888) may also be identical to Bresadola (1928). *Melanoleuca brevipes* as described by Gillman and Miller (1977) is characterised by a brown to dark brown pileus, grey-brown lamellae, the presence of 1-celled or 2-celled cheilocystidia and by the absence of pleurocystidia. However, the context is described as white; thus, it probably represents a different species.

According to Bon (1990a), the closest taxa to his "true" *M. brevipes* are *M. subbrevipes* Métrod ex Bon differing by very robust and fleshy basidiocarps, *M. malenconii* Bon with a dark fuligineous brown (sometimes almost blackish or dark grey) pileus, *M. diverticulata* Moreno & Bon, and *M. subexcentrica* Bon, both with a pale grey or beige pileus. However, results of DNA studies of the holotypes of *M. diverticulata* and *M. subexcentrica* (not published) have showed their identity with *M. rasilis* (Fr.) Singer in the sense of Vizzini et al. (2011). Boekhout (1988, 1999) differentiated *M. brevipes* from *M. grammopodia* only by the medium-sized basidiocarps, less distinctly fibrillose stipe and brown stipe base context of the former. In his key, Vesterholt (2012) placed *M. brevipes* next to *M. subbrevipes* Bon s.l., but the only differences are the more robust and fleshy basidiocarps of *M. subbrevipes*. According to the information mentioned above, it is evident that the concepts of *M. brevipes* by various authors are in disagreement.

The original table (Bulliard 1791) certainly does not represent *M. grammopodia* for the coloured context in the stipe base, but this character is in accordance with *M. humilis* and *M. malenconii*. However, Bulliard (1791) described the stipe of *M. brevipes* as "stipite … nudo, brevissimo…" which excludes *M. malenconii*. Bresinsky and Stangl (1977) characterised *M. brevipes* as a spring (April to May) fungus, which is also in accordance with the phenology of *M. humilis* in both Central and South Europe—it starts to grow in April and grows until autumn (November; Vizzini et al. 2012). See "Specimens examined" sections for details. We are not able to synonymise *M. brevipes* with any discussed taxon with certainty. Therefore, as a conclusion of our results, we consider *M. brevipes* a nomen dubium.

A detailed discussion on the identity of *M. humilis* in older mycological literature was published by Vizzini et al. (2012). *Melanoleuca malenconii* Bon (= *M. sublanipes* Fontenla et al.), the closest species, differs by having paler, less robust basidiocarps with a paler coloured stipe, which is pruinose-pubescent to distinctly floccose (apex) and sometimes floccose-hairy (especially in the lower part), and by its DNA sequences (Vizzini et al. 2012, this study). Moreover, *M. malenconii* is a (late) autumn species growing mostly from the second half of October (Vizzini et al. 2012, this study). Métrod (1948) mentioned that descriptions of *M. humilis* in various literature are in disagreement concerning the stipe shape (bulbous, attenuated) and lamellae (narrow, ventricose); his concept agrees with that by Ricken (1915: tab. 96, 2) and our opinion. In contrast, Watling and Turnbull (1998) described a whitish context in the stipe base of *M. humilis*, so that this species probably represents another taxon. *Melanoleuca humilis* is omitted in several monographic *Melanoleuca* studies (e.g., Kühner 1978; Boekhout 1999; Caroti et al. 2006; Vesterholt 2012). In their descriptions of *M. humilis*, a range of authors mention smaller basidiospores in comparison with our data (e.g., Métrod 1948: 8–8.5×4.5–5.5 μm; Bresinsky and Stangl 1977: 7–8×(4.5)5–5.5 μm). Our results support Métrod's opinion.

Melanoleuca grammopodia is mostly characterised as a fungus with a large pileus and a long and slender stipe (Watling and Turnbull 1998; Caroti et al. 2006; Vesterholt 2008, 2012). Bresinsky and Stangl (1977) mentioned that the stipe may be distinctly longer than the pileus diameter in young basidiocarps and slightly shorter in older ones. The short-stiped form is sometimes considered a separate taxon [*M. subbrevipes* Métrod ex Bon, *M. grammopodia* var. *subbrevipes* (Métrod) Kühner & Romagn. (nom. inv.), *M. grammopodia* f. *subbrevipes* (Métrod) L. Remy (nom. inv.)] (Kühner 1978; Watling and Turnbull 1998; Caroti et al. 2006). Originally, *M. subbrevipes* was described invalidly (lacking a Latin diagnosis) for a fungus with an up to 150(200) mm broad, rather uniformly ochraceous grey-coloured pileus with a fulvous brown centre, ochraceous grey or white cream coloured lamellae, a short, (50)60–80(100)× 10–20(30) mm stipe, and a ± brownish context in the stipe (Métrod 1942). Later, Bon (1990b) validated the name as *M. subbrevipes* Métrod ex Bon. The holotype material

(Crans–Jura, September 1935, PC 0096642; rather poor and difficult to revise) was revised by R. Fontenla and R. Para; its basidiospores agreed more or less with those of *M. humilis*; however, they did not find cheilocystidia due of the bad condition of the holotype (Fontenla and Para pers. comm.). Therefore, the identity of the holotype specimen cannot be confirmed. We studied authentic material identified by M. Bon (the author of the validation) as *M. subbrevipes*, and found it to agree both microscopically and phylogenetically with *M. grammopodia*. However, we refrain to consider this taxon a short-stiped form of *M. grammopodia*, due to impossibility to revise the stipe context colour (white in *M. grammopodia*, brownish in the original description of *M. subbrevipes*) in the type specimen, which is in bad condition. No valid combination of the name *subbrevipes* at the infraspecific level (variety, form) of *M. grammopodia* has been made to date.

A distinctly short-stiped form of *M. grammopodia* was photographed by Paoletti et al. (2008) and synonymised with *M. subbrevipes*. However, their fungus has a rather vividly brown-ochraceous or yellowish grey-brown pileus, and its exact identity to *M. grammopodia* specimens studied by us is not certain. Boekhout (1988, 1999) synonymised *M. grammopodia* and *M. subbrevipes*, and proposed *M. grammopodia* f. *macrocarpa* Boekhout for a short-stiped form with a large pileus. However, this new form phylogenetically belongs to *M. humilis* (Fig. 1). Nevertheless, as also confirmed by our results, *M. grammopodia* may also have large forms. The ITS sequence of specimen SLO 1469 with an up to 260 mm wide pileus proved to be *M. grammopodia*. The most important discriminating character between large forms of *M. grammopodia* and *M. humilis* remains the colour of the context in the stipe base.

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References

Antonín V, Sedlák P, Tomšovský M (2013) Taxonomy and phylogeny of European *Gymnopus* subsection *Levipedes* (Basidiomycota, *Omphalotaceae*). Persoonia 31:179–187. doi:10.3767/003158513X674043

Antonín V, Rimóczi I, Benedek L, Papp V, Szarkándi JG, Bálint D, Nagy LG, Papp T, Ďuriška O, Tomšovský M (2014) *Melanoleuca juliannae* (Basidiomycota, *Tricholomataceae*), a new species from subgen. *Urticocystis*. Phytotaxa 170(1):13–23. doi:10.11646/phytotaxa.170.2.2

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

Boekhout T (1999) *Melanoleuca* Pat. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds) Flora agaricina neerlandica, vol 4. AA Balkema, Rotterdam, pp 153–165

Bon M (1978) Tricholomataceae de France et d'Europe occidentale (sous-famille *Leucopaxilloideae* (Singer) Bon). Doc Mycol 9(33):1–79

Bon M (1990a) Les Tricholomes et ressemblant. Doc Mycol Mém Hors Sér 2:1–163

Bon M (1990b) Novitates – Combinaisons nouvelles et validations. Doc Mycol 20(78):37–40

Bon M (1990c) Novitates – Taxons nouveaux et validations. Doc Mycol 20(79):57–62

Bresadola J (1928) Iconographia mycologica, vol 3. A. Ingegnoli, Mediolani

Bresinsky A, Stangl J (1977) Beträge zur Revision M. Britzelmayrs "Hymenomyceten aus Südbayern" 13. Die Gattung *Melanoleuca* unter besonderer Berücksichtigung ihrer Arten in der Umgebung von Augsburg. Z Pilzk 43:145–173

Bulliard P (1791) Herbier de la France, vol 11. Didot, Debure, Belin, Paris

Caroti V, Consiglio G, Contu M, Fontenla R, Gottardi M, Para R (2006) Contributio alla conoscenza dei macromiceti dell'Emilia-Romagna. XXII. Genere Melanoleuca. Boll AMER 22(67):9–39

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9(8):772

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36(suppl 2):W465–W469. doi:10.1093/nar/gkn180

Esteve-Raventós F, Llistosella Vidal J, Ortega Diaz A (2007) Setas de la Peninsula Ibérica e Islas Baleares. Ediciones Jaguar, Madrid

Fontenla R, Para R (2013) Observations on *Melanoleuca*. On some of Bresadola's *Tricholoma* referable to *Melanoleuca*. Bresadoliana 1(2010):35–62

Fontenla R, Gottardi M, Lavorato C, Para R (2003) Osservazioni sul genere *Melanoleuca*: Tre specie descritte da Malençon & Bertault. Rev Micol 46:337–350

Fries EM (1821) *Systema mycologicum*. E. Mavritius, Gryphiswaldiae

Gillman LS, Miller OK Jr (1977) A study of the boreal, alpine and arctic species of *Melanoleuca*. Mycologia 69:927–951

Gröger F (2006) Bestimmungsschlüssel für Blätterpilze und Röhrlinge in Europa, Teil 1. Regensb Mykol Schriften 13:1–638

Huelsenbeck JP, Ronquist F (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. doi:10.1093/bioinformatics/btg180

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. doi:10.1093/molbev/mst010

Konrad P, Maublanc A (1924–1933) Icones selectae fungorum, vol 3. Paul Lechevalier, Paris

Kornerup A, Wanscher JH (1983) Methuen handbook of colour, 3rd edn. Eyre Methuen, London

Kühner R (1978) Agaricales de la zone alpine. Genre *Melanoleuca* Pat. Bull Soc Linn Lyon 47(1):12–52

Malençon G, Bertault R (1975) Flore des champignons supérieurs du Maroc. Tome II. Trav Inst Sci Chérifien 33:1–540

Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Mol Phylog Evol 35(1):1–20. doi:10.1016/j.ympev.2004.11.014

Métrod G (1942) Les Tricholomes. Rev Mycol Suppl 7:89–96

Métrod G (1948) Essai sur le genre *Melanoleuca* Patouillard emend. Bull Soc Mycol France 64:141–165

Nikolcheva LG, Bärlocher F (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. Mycol Progr 3:41–49. doi:10.1007/s11557-006-0075-y

Paoletti B, Papetti C, Zovadelli C (2008) Escursioni di studio alla Fattoria didattica "Catena Rossa", Cagnaghe di Sarezzo V.T. – II. Boll Circ Micol G Carini 56:1–32

Quélet L (1888) Flore mycologique de la France et des pays limitrophes. O Doin, Paris

Rambaut A, Drummond AJ (2009) Tracer version 1.5 [computer program]. http://beast.bio.ed.ac.uk

Ricken A (1915) Die Blätterpilze. TO Weigel, Leipzig

Sánchez-García M, Cifuentes-Blanco J, Matheny PB (2013) Revisión taxonómica del género *Melanoleuca* en México y descripción de especies nuevas. Rev Mex Biodiv 84:S111–S127. doi:10.7550/rmb.31569

Singer R (1935) Étude systématique sur les *Melanoleuca* d'Europe et clé des espèces observes en Catalogne. Cavanillesia 7:122–132

Singer R (1943) Das system der Agaricales. III. Ann Mycol 41:1–189

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer, Sunderland

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Thiers B (2014, continuously updated) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/. Accessed: 3 July 2015

Tomšovský M (2012) Delimitation of an almost forgotten species *Spongipellis litschaueri* (Polyporales, Basidiomycota) and its taxonomic position within the genus. Mycol Progr 11:415–424. doi:10.1007/s11557-011-0756-z

Vesterholt J (2008) Munkehat (*Melanoleuca*) i Danmark. Svampe 57:18–29

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

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. doi:10.5248/118.361

Vizzini A, Ercole E, Fontenla R, Para R (2012) Osservazioni sul genere *Melanoleuca*. *M. humilis* e *M. sublanipes*. Micol Veget Med 27:93–108

Watling R, Turnbull E (1998) *Cantharellaceae*, *Gomphaceae* and amyloid-spored and xeruloid members of *Tricholomataceae* (excl. *Mycena*). In: Henderson DM, Orton PD, Watling R (eds) British Fungus Flora Agarics and Boleti 8:1–189

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic, New York