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Taxonomy, ecology and distribution of *Melanoleuca strictipes* (Basidiomycota, *Agaricales*) in Europe

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Melanoleuca strictipes (P. Karst.) Métrod, a species characterised by whitish colours and macrocystidia in the hymenium, has for years been identified as several different species. Based on morphological studies of 61 specimens from eight countries and a phylogenetic analysis of ITS sequences, including type material of *M. subalpina* and *M. substrictipes* var. *sarcophyllum*, we confirm conspecificity of these specimens and their identity as *M. strictipes*. The lectotype of this species is designated here. The morphological and ecological characteristics of this species are presented.

Key words: taxonomy, phylogeny, *M. subalpina*, *M. substrictipes* var. *sarcophyllum*.

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Tmavobělka světlemedová – *Melanoleuca strictipes* (P. Karst.) Métrod, charakterizovaná bělavým zbarvením plodnic a přítomností makrocystid v hymeniu, byla v minulosti uváděna pod jménem několika odlišných druhů. Na základě morfologických studií 61 položek sbíraných v osmi zemích a fylogenetické analýzy sekvencí ITS, včetně typového materiálu *M. subalpina* **a** *M. substrictipes* var. *sarcophyllum*, jsme potvrdili konspecificitu těchto položek a jejich identitu s *M. strictipes*. Je rovněž vybrán lektotyp pro tento druh. V článku je publikována morfologická a ekologická charakteristika tohoto druhu.

INTRODUCTION

Melanoleuca Pat. is a well-delimited genus of agaricoid basidiomycetes, currently assigned to suborder *Pluteineae* (Dentinger et al. 2015). Basidiomata are characterised by a collybioid to tricholomatoid habit; cap and stipe colours are less distinct, mostly in shades of brown and grey; lamellae are emarginate and narrowly or broadly adnate, rarely shortly decurrent, mostly white, whitish to grey or brownish. The combination of a number of microscopic characters, ellipsoid hyaline basidiospores covered with amyloid ornamentation, absence of clamp connections, and specific shapes of cheilocystidia and pleurocystidia, make *Melanoleuca* clearly distinguishable from other genera.

However, the identification of species within the genus is rather problematic, due to the high macro- and micromorphological variability and overlaps of identifying features in many species. Moreover, numerous species described in history are difficult to define. According to Vizzini et al. (2011), there are 332 validly published names (species and infraspecific taxa) in this genus, including 126 European and 206 extra-European taxa. However, most of these published names are treated as synonyms.

In 2012 we started a taxonomic revision of *Melanoleuca* using both morphological and molecular methods. DNA sequence data have been very helpful to elucidate some taxonomical problems. Based on our research (Ďuriška et al. 2013a, 2013b, Antonín et al. 2014, Ďuriška et al. 2015, Antonín et al. 2015), we have confirmed the occurrence of 28 species and infraspecific taxa in Europe to date.

During our molecular screening of European *Melanoleuca* specimens, using DNA sequences of the internal transcribed spacers of the ribosomal RNA gene (ITS) and morphological analyses, we recognized just one species typical by whitish coloured basidiomata and well-developed macrocystidia, occurring in Europe – *Melanoleuca strictipes* (P. Karst.) Métrod.

The principal aim of this study is to solve the phylogenetic position and taxonomy of this species and related taxa. Macro-, micromorphological and ecological data of the species are presented here.

MATERIAL AND METHODS

Studied specimens. We studied 61 specimens from eight countries: Austria, Czech Republic, France, Germany, Italy, Romania, Slovakia and Slovenia, including the type material of *M. substrictipes* Kühner (holotype), *M. substrictipes* var. *sarcophyllum* (Kühner) Fontenla, Para & Vizzini (holotype) and *M. subalpina* (Britzelm.) Bresinsky & Stangl (epitype). All specimens used in this study are listed in Results, and herbarium abbreviations follow Thiers (on-line). The

herbarium of R. Para and R. Fontenla is abbreviated as „herb. R.P.–R.F.“. Authors of fungal names are cited following the Authors of Fungal Names website (http://www.indexfungorum.org/AuthorsOfFungalNames.htm).

Morphology. Macroscopic descriptions of collected specimens are based on fresh basidiomata. Colour abbreviations are according to Kornerup & Wanscher (1983). Microscopic features are described from dried material mounted in KOH (approx. 10% solution), Melzer's reagent, and Congo Red, using Olympus BX-50 and Leica DM 1000 light microscopes with a magnification of 1000×. For basidiospores, the sizes are given as minimum, maximum (in parentheses) and average values, and 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).

Molecular methods. DNA was isolated from dried fungal material and the ITS region was amplified according to Antonín et al. (2015). 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 used for amplification. The sequences were aligned using the MAFFT, version 7 online program, setting up the Q-INS-i option (Katoh & Toh 2008).

The dataset was supplemented with sequences published by Vizzini et al. (2011), Sánchez-García et al. (2013), and Yu et al. (2014). The aligned ITS dataset was 670 bp long (528 conserved, 123 variable, and 53 singleton positions as determined in the MEGA 6 program). The ITS sequences of *Melanoleuca* spp. from subgenus *Urticocystis* were selected as an outgroup.

Phylogenetic analysis. Maximum likelihood (ML) and Bayesian inference (BI) analyses were used to estimate phylogenetic relationships. The BI analysis was conducted with MrBayes 3.2.6 (Ronquist et al. 2012). Likelihood settings from the best-fit model for ITS data (TPM2uf+ G**)** were selected by the Akaike information criterion in jModelTest2 (Darriba et al. 2012). We ran four chains for 10 million generations. The burn-in value was set to 2.5 million generations. Sampling frequency was set to every 1000th generation.

ML analyses were performed with RAxML-HPC v. 8 (Stamatakis 2014) with the GTRCAT model of evolution. Branch support was determined by non-parametric bootstrapping (BS) with the rapid bootstrapping option setting the number of replicates automatically (456 replicates).

RESULTS

PHYLOGENY

The specimens used in the molecular analyses are summarized in Tab. 1. The phylogenetic analyses (Fig. 1) resulted in a well-supported clade of *Melanoleuca strictipes* including a lectotype of *M. substrictipes*, holotype of *M. substrictipes* var. *sarcophyllum*, and epitype of *M. subalpina*. *Melanoleuca strictipes* is clearly separated from *M. cinereifolia*, *M. communis* and *M. leucopoda*.

Specimens identified as *M. nivea* occupy different positions on the tree, therefore the identity of the species is unclear. Moreover, two other clades include sequences under various names: *M. albifolia*, *M. atripes*, *M. friesii* and *M. polioleuca* in one clade, and *M. arcuata*, *M. heterocystidiosa* and *M. nivea* in the other one. This supports the unclear identity of several traditional names or common misidentifications of specimens in *Melanoleuca*.

Tab. 1. List of *Melanoleuca* sequences generated for this study.

Species	Country	Specimen no.	GenBank accession number of ITS sequences
M. cinereifolia (identified as *M. strictipes*)	France	RC/F88135, LIP	KY417110
M. nivea	Belgium	Bon 92209, LIP	KY417111
M. strictipes	Czech Republic	BRNM 781382	KY417093
M. strictipes	Czech Republic	BRNM 781381	KY417094
M. strictipes	Slovakia	BRNM 781386	KY417095
M. strictipes	Czech Republic	BRNM 781384	KY417096
M. strictipes	Czech Republic	BRNM 781385	KY417097
M. strictipes	Czech Republic	BRNM 737301	KY417098
M. strictipes	Slovakia	SLO 1585	KY417099
M. strictipes	Austria	Dvořák 46/06, BRNU 634658	KY417100
M. strictipes	Austria	Dvořák 47/06, BRNU 634659	KY417101
M. strictipes	Austria	Dvořák 53/06, BRNU 634660	KY417102
M. strictipes	Slovakia	BRNM 781380	KY417103
M. strictipes	Italy	BRNM 781383	KY417104
M. strictipes	Slovakia	BRNM 781410	KY417108
M. strictipes	Slovakia	SLO 1660	KY417109
M. substrictipes var. *sarcophyllum*	France	G K65-24, holotype	KY417106
M. substrictipes	France	G K66-13, lectotype, part B	KY417105
M. subalpina	Germany	M-0140061, epitype	KY417107

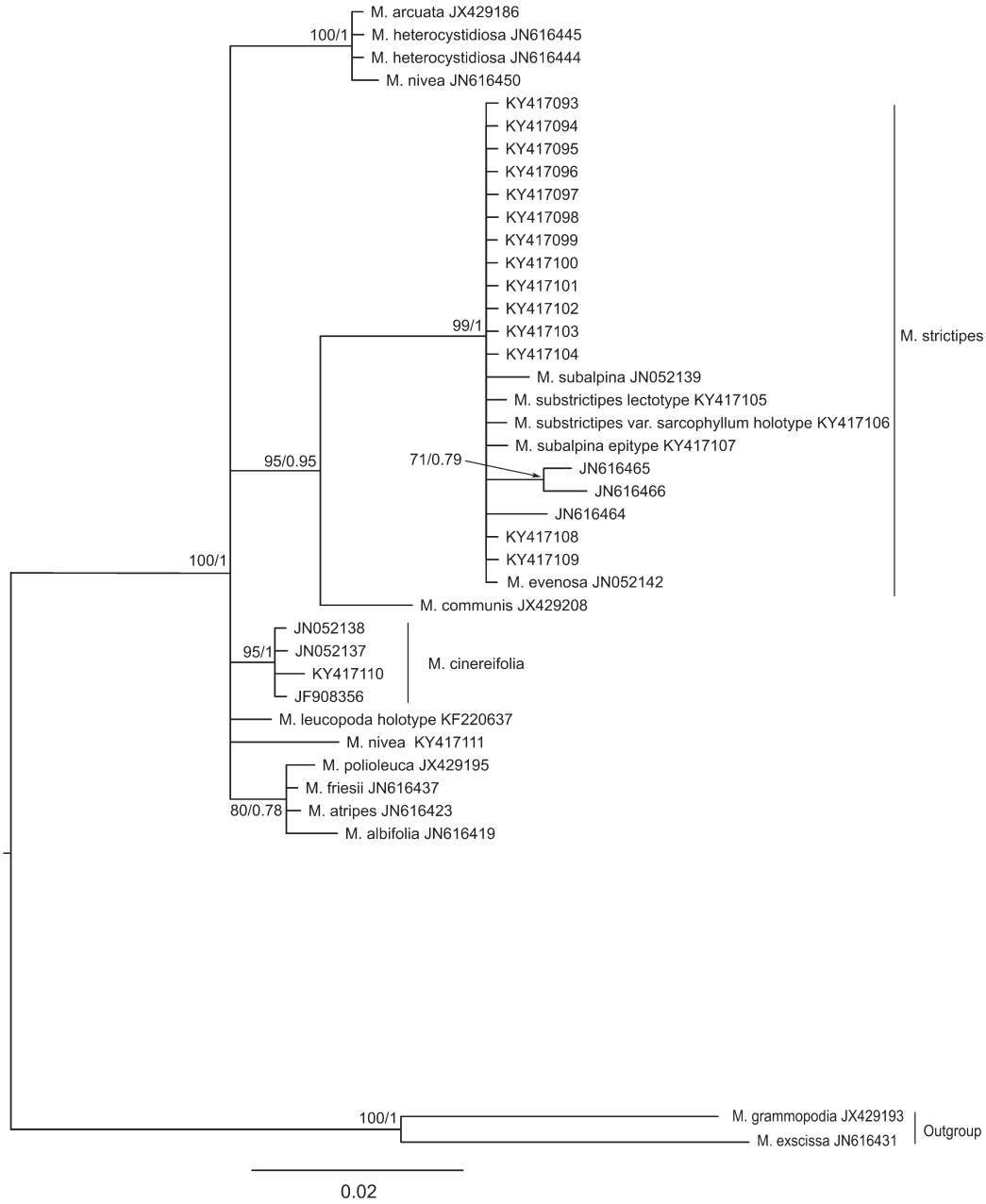

Fig. 1. Phylogenetic tree of *Melanoleuca strictipes* inferred from Bayesian analysis of ITS sequences. For legends to newly generated sequences, see Tab. 1. Support values (ML bootstrap support/Bayesian posterior probabilities) are given at the branches. The bar indicates the number of expected substitutions per position.

TAXONOMY

Melanoleuca strictipes (P. Karst.) Métrod, Bulletin de la Société Mycologique de France 64: 160, 1948 Figs. 2–6

≡ *Tricholoma strictipes* P. Karst., Meddelanden af Societatis pro Fauna et Flora Fennica 6: 7, 1881. – *Collybia strictipes* (P. Karst.) Sacc., Sylloge Fungorum 9: 30, 1891. – *Agaricus strictipes* (P. Karst.) Britzelm., Bericht des Naturhistorischen Vereins in Augsburg 31: 160, 1894. – *Melanoleuca evenosa* var. *strictipes* (P. Karst.) Pace, L'atlante dei funghi: 155, 1975 (comb. inv.).

= *Tricholoma cnista* Fr. ss. Bres., Fungi Tridentini: 44–45, 1881.

= *Tricholoma cnista* var. *evenosum* (Sacc.) Sacc. & Traverso, Sylloge Fungorum 20: 992, 1911. – *Tricholoma evenosum* (Sacc.) L. Maire, Étude Synthètique sur le Genre *Tricholoma*: 32, 1916. – *Melanoleuca evenosa* (Sacc.) Konrad, Bulletin de la Société Mycologique de France 43: 184–186, 1927.

= *Agaricus subalpinus* Britzelm., Hymenomycetes aus Südbayern 8: 4, 1894. – *Tricholoma subalpinum* (Britzelm.) Sacc., Sylloge Fungorum 11: 12, 1895. – *Melanoleuca subalpina* (Britzelm.) Bresinsky & Stangl, Beihefte zur Zeitschrift für Pilzkunde 1: 46, 1976.

= *Melanoleuca substrictipes* Kühner, Bulletin de la Société Linnéenne de Lyon 47(1): 52, 1978 (pro parte, see notes below).

= *Melanoleuca substrictipes* var. *sarcophylla* Kühner in Bon, Flore Mycologique d'Europe 2: 125, 1991. – *Melanoleuca exscissa* f. *sarcophylla* (Kühner) Fontenla, Para & Vizzini, Mycotaxon 118: 374, 2011.

Lectotype (designated here). *Tricholoma strictipes* P. Karst., Finland, Etelä-Häme, Tammela, Mustiala, Salois, July 1878 leg. et det. P.A. Karsten (H 6003412, herbarium Petter Adolf Karsten).

Macroscopic characters. Pileus (30)55–115 mm broad, low convex to applanate, slightly depressed and with distinct to indistinct broad umbo at centre, slightly inflexed to straight at margin, irregular, hygrophanous, smooth or finely rugulose (under lens), glabrous, (ochraceous) brown (5B3, 6C-D5-6) at centre; paler, leathery yellow (± 5A2-3) to almost cream (3A2-3) coloured at margin. Lamellae moderately close, L = 50–100, l = (2)3–6, irregular, emarginate and attached with tooth, sinuate, sometimes rather narrow (up to 6 mm), cream coloured with beige tint, with concolorous, finely pubescent edge; slightly yellowing when drying out. Stipe (40)60–130 × 7–11 mm, cylindrical, slightly broadened at apex, with a distinct bulb (up to 20 mm) at base, longitudinally fibrillose, slightly twisted, finely pubescent-tomentose at apex, white or whitish; with white basal mycelium. Context whitish, ochraceous brownish under pileipellis, with fungoid, sometimes slight spermatic smell and mild taste.

Microscopic characters. Basidiospores 7–10 × (4)4.5–6 µm, average = 9.1 × 5.2 µm, E = 1.45–2.10, Q = 1.75, ellipsoid, ellipsoid-fusoid or ellipsoid-cylindrical, ornamentation minute, verruculose with connections, amyloid. Basidia (19)23–36 × 9–12 µm, 4-spored, rarely 2-spored, clavate. Basidioles 15–33 × 5–11 µm, clavate, subcylindrical, subvesiculose. Cheilo- and pleurocystidia macrocystidioid, (18)46–85(90) × (9)15–21(28) µm, fusoid, sublageniform, sometimes almost lanceolate, obtuse, rarely septate, thin-walled with often slightly thick-

Fig. 2. *Melanoleuca strictipes*, Staré Hamry – Jamník, Moravskoslezské Beskydy Mts., Czech Republic, 24 July 2011 (BRNM 737301). Photo V. Antonín.

Fig. 3. *Melanoleuca strictipes*, Someşu Rece, Gilăului Mts., Romania, 4 Oct. 2014 (SLO 1661). Photo S. Jančovičová.

Fig. 4. *Melanoleuca strictipes*, Pianalunga near Alagna Valsesia, Monterosa, Italy, 27 Aug. 2014 (BRNM 781383). Photo V. Antonín.

Fig. 5. *Melanoleuca strictipes*, Pianalunga near Alagna Valsesia, Monterosa, Italy, 27 Aug. 2014 (BRNM 781383). Photo H. Ševčíková.

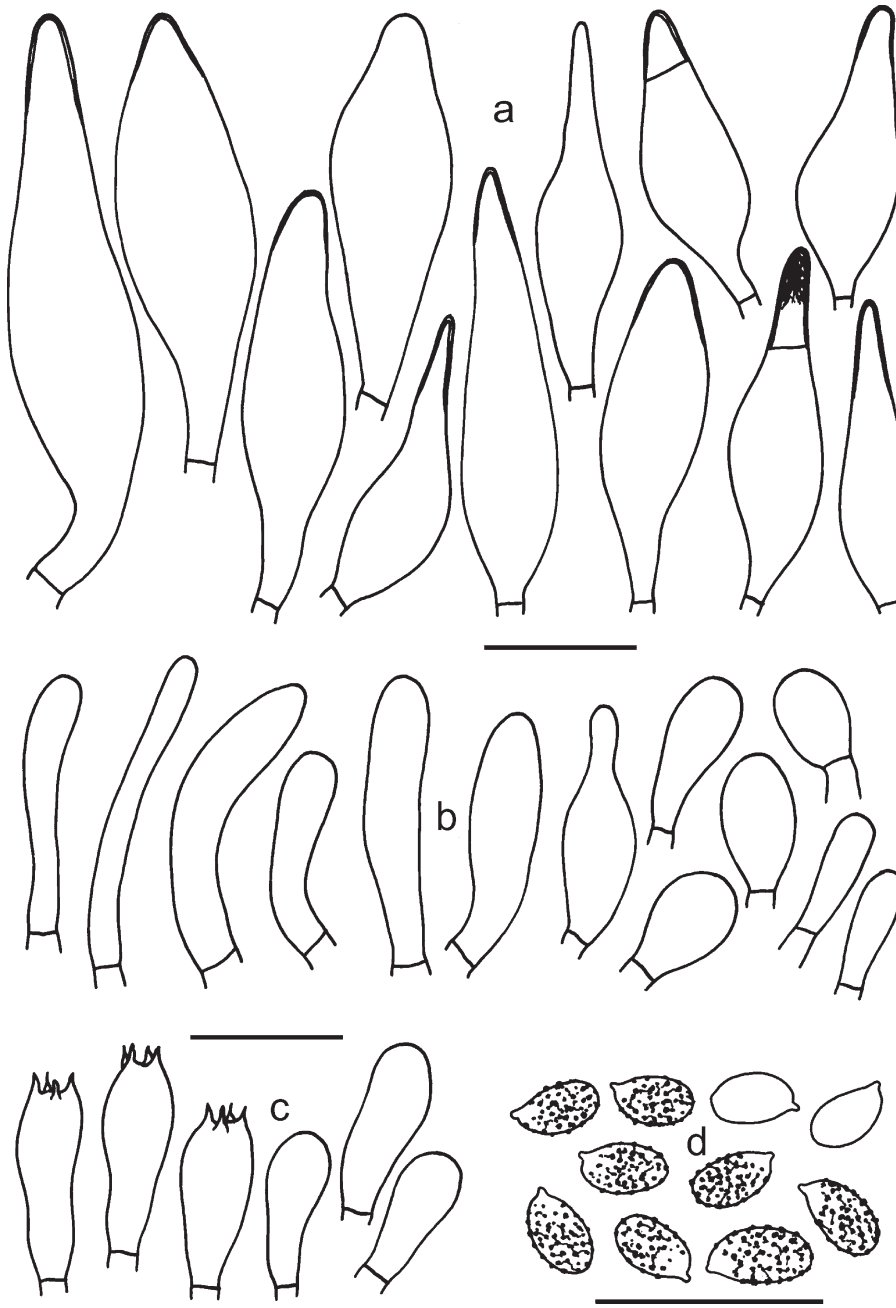

Fig. 6. Microscopic characters of *Melanoleuca strictipes* (BRNM 781384, BRNM 781383, BRNM 781380): **a** – cheilocystidia and pleurocystidia; **b** – caulocystidia; **c** – basidia; **d** – basidiospores. Scale bars = 20 µm. Del. S. Jančovičová.

walled apex, muricate or not. Trama hyphae cylindrical to subinflated, thin-walled, non-dextrinoid, up to 15(25) µm wide. Pileipellis an ixocutis (up to subixotrichoderm at centre), composed of cylindrical, thin-walled, smooth or minutely incrusted, non-dextrinoid, 3–8 µm wide hyphae; terminal cells appressed to erect, cylindrical, narrowly clavate, (sub)fusoid, thin-walled, up to 10 µm wide. Stipitipellis a cutis of cylindrical, parallel, ± thin-walled, non-dextrinoid, up to 7 µm wide hyphae. Caulocystidia of two types: (1) frequent, 18–48 × 4–12(14) µm, clavate, cylindrical, subfusoid, rarely irregular, branched or subcoralloid, thin-walled, and (2) macrocystidia, scattered, 59–73 × 13–18 µm, fusoid or sublageniform, thin-walled. Basal mycelium of cylindrical, ± thin-walled, up to 8 µm wide hyphae; mycelial cystidia very rare, 45–52 × 4–4.5 µm, subulate. Clamp connections absent.

Ecology. On soil, in grass on montane to alpine (snow-patch) grassland habitats and pastures, in forest margins (*Picea*, *Betula*), and in broadleaved forests (*Quercus*).

Distribution. *Melanoleuca strictipes* is widely distributed throughout Europe (e.g. our studies, Bon 1991, Legon & Henrici 2005, Vesterholt 2012).

Specimens examined

Melanoleuca strictipes

Austria. Carinthia. High Tauern, Innerfragant, near the road to Fraganter Schützhaus, at edge of spruce forest, 3 July 2006 leg. E. Hettenbergerová (Dvořák 46/06, BRNU 634658). – Ibid., near the road above Fraganter Schützhaus, alpine pasture, 4 July 2006 leg. D. Dvořák 47/06 (BRNU 634659). – Ibid., mountain ridge W of Fraganter Schützhaus, near the Bretterich peak (2312 m), alpine grassland, 5 July 2006 leg. D. Dvořák 53/06 (BRNU 634660).

Czech Republic. Moravia. Moravskoslezské Beskydy Mts., Staré Hamry – Jamník, alt. 700–750 m, 29 July 2000 leg. V. Antonín 00.37 & D. Janda (BRNM 781382). – Ibid., 24 July 2011 leg. V. Antonín 11.62 & D. Janda (BRNM 737301). – Moravskoslezské Beskydy Mts., Horní Lomná, Mionší National Nature Reserve, Polana, alt. 870–890 m, 24 Aug. 2014 leg. D. Dvořák 140824-70 (BRNU 634652). – Rozdrojovice, "Obora" game preserve, alt. c. 400 m, 1 June 2006 leg. V. Kotek (BRNM 781381). – Bílé Karpaty Mts., Nedašov, Hrušovská dolina valley, site called Lázce, alt. 630–650 m, 5 Sept. 2010 leg. V. Antonín 10.303 and 10.308 (BRNM 781384, BRNM 781385). – Hodonín, Hodonínská doubrava, alt. c. 190 m, 15 May 1986 leg. H. Vlčková (BRNM 383942). – Ibid., site called "U Cvingrů", alt. c. 190 m, 11 July 2013 leg. H. Ševčíková (BRNM 751690).

France. Rhône-Alpes. Savoie, Parc Nat. de la Vanoise, vicinity of Pralognan, between le Pas de l'Ane and le Cirque des Nants, alt. 1900 m, 20 July 1966, R. Kühner K66-13 (G, part B of the holotype of *M. substrictipes*, see notes below). – Savoie, Parc National de la Vanoise, vicinity of Pralognan, Hameau des Prioux, alt. 1700 m, 21 Aug. 1965 leg. et det. R. Kühner (G K 65-24, holotype of *M. substrictipes* var. *sarcophyllum*). – Drôme Dept., Bouvante, Chaud Clapier, alt. c. 1400 m, 31 May 2015 leg. T. Bruyère (BRNM 781410).

Germany. Bavaria. Between Roßhaupten and Seeg near Vordersulzberg, 19 May 1963 leg. A. Bresinsky (M 0140061, epitype of *M. subalpina*; Fontenla & Para 2008).

Italy. Piemont. Cuneo, Garessio 2000 di Colla di Casotto, in *Fagus sylvatica* forest, alt. c. 1200 m, 16 Sept. 2005 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 050916-01, ANC M0172). – Vercelli,

Monterosa, Alagna Valsesia, Pianalunga, alt. 2200 m, 27 Aug. 2014 leg. V. Antonín (14.126) & H. Ševčíková (BRNM 781383). – Vercelli, Monterosa, Alagna Valsesia, Val d'Olen, on pasture, alt. c. 2300 m, 27 Aug. 2008 leg. M. Filippa (herb. R.P.–R.F. 080827-08, ANC M0171). – Ibid., 29 Aug. 2008 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 080829-01, ANC M0173). – Ibid., 27 Aug. 2008 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 080827-03, 080827-04). – Ibid., 29 Aug. 2008 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 080829-02). – Vercelli, Pila, Failungo, in *Tilia cordata* forest along the river Sesia, alt. c. 800 m, 26 Aug. 2005 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 050826-01, 050826-02). – Ibid., 28 Aug. 2008 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 080828-01). – Lombardy. Lecco, Casargo, Alpe di Giumel, on pasture, alt. 1500 m, 6 June 2003 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 030606-01). – Lecco, Taceno, Bosco di Ciarello, in *Picea abies* forest, alt. 650 m, 6 June 2003 leg. D. Bolognini & G. Baiano (herb. R.P.–R.F. 030606-02). – Lecco, Vendrogno, Betuleto di Camaggiore, on pasture, alt. 1150 m, 7 June 2003 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 030607-01). – Sondrio, Valdisotto, Bormio 2000, on pasture, alt. 1900 m, 7 Sept. 2001 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 010907-01). – Sondrio, Valdisotto, Le Mosse, on pasture, alt. 1430 m, 4 Sept. 2001 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 010904-01). – Bergamo, Gromo, Valle di Goglio, on meadow, alt. 1200 m, 26 Aug. 2002 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 020826-01). – Brescia, Brenno, Piana del Gaver, on pasture with *Alnus viridis*, 17 July 2004 leg. M. Panchetti (herb. R.P.–R.F. 040717-01). – Trentino. Ala, Malga Cornafessa, edge of *Fagus sylvatica* forest, alt. 1000 m, 30 June 2007 leg. A. Aiardi (herb. R.P.–R.F. 070630-01). – Daone, Bissina Lake, on pasture, alt. 1200 m, 12 Sept. 2002 leg. P. Bigoni (herb. R.P.–R.F. 020912-02). – Pejo, Lago Careser, on pasture, alt. 2500 m, 1 Sept. 2000 leg. E. Carassai (herb. R.P.–R.F. 000901-01). – Tesero, Stava, 17 Sept. 2009 leg. F. Bersan (herb. R.P.–R.F. 090917-02). – South Tyrol. Braies, Prato Piazza, on pasture, alt. 2000 m, 29 July 2005 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 050729-01, ANC M0239). – Brennero, Malga Zirago, on pasture, alt. 1900 m, 23 July 2004 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 040723-01, 040723-02, 040723-05). – Ibid., 24 July 2004 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 040724-01). – Dobbiaco, Franadega, on pasture, alt. 1600 m, 21 July 2005 leg. A. Bonometti (herb. R.P.–R.F. 050721-01). – Dobbiaco, Valle San Silvestro, edge of pathway, alt. 1300 m, 23 July 2005 leg. L. Fracalossi (herb. R.P.–R.F. 050723-02). – Racines, Calice, on pasture, alt. 1600 m, 24 July 2004 leg. D. Ferri (herb. R.P.–R.F. 040724-02). – Racines, Telves, on pasture, alt. 1400 m, 22 July 2004 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 040722-01). – Stelvio, Sentiero Natura di Solda, 2 Aug. 2006 leg. F. Bellù (herb. R.P.–R.F. 060802-01, 060802-02, 060802-03). – Stelvio, Trafoi, 3 Aug. 2006 leg. R. Dalla Torre (herb. R.P.–R.F. 060803-01). – Stelvio, Tre Fontane, in *Picea abies* forest, near the road, 26 July 2012 leg. A. Arcangeli & G. Bistocchi (herb. R.P.–R.F. 120726-01). – Pfitsch (Vizze), Pfitschtal (Val di Vizze), Pfitschjoch, alt. 2260–2280 m, 29 July 2016 leg. F. Cecconi (BRNM 781303). – Val di Vizze, St. Jacob, on pasture, alt. 1500 m, 23 Aug. 2007 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 070823-02). – Val di Vizze, Stain, 23 Aug. 2007 leg. W. Tommasi (herb. R.P.–R.F. 070823-07). – Valle Aurina, on pasture along a pathway, alt. 1650 m, 25 July 2005 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 050725-01). – Vipiteno, Punta Gallina, on pasture, alt. 2400 m, 22 July 2004 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 040722-03). – Veneto. Belluno, Tambre d'Alpago, Val Menera, 24 May 2008 leg. A. Fontenla (herb. R.P.–R.F. 080524-01). – Emilia-Romagna. Parma, Albareto, Passo Cento Croci, on pasture, alt. 1055 m, 8 June 2012 leg. R. Jon (herb. R.P.–R.F. 120608-01, 120608-03). – Calabria. Cosenza, Acri, Bosco Dominiello, edge of pathway near *Quercus cerris* and *Pinus calabrica*, alt. 800 m, 5 Oct. 2004 leg. C. Lavorato (herb. R.P.–R.F. 041005-31).

Romania. Gilăului Mts., Someşu Rece, alt. 750 m, 4 Oct. 2014 leg. S. Jančovičová (SLO 1661, SLO 1662, SLO 1663). – Maramures Mts., Kirlibaba, near confluence of the Bistrita and Tibau rivers, alt. c. 1000 m, 28 June 1986 leg. V. Antonín (BRNM 462111).

Slovakia. Záhorská nížina, Šaštín, Jubilejný les, water-logged meadow, alt. 200 m, 8 Aug. 2014 leg. I. Tomášeková (SLO 1660). – Západné Tatry Mts., Zuberec, saddle under Mt. Osobitá, mountain pasture, alt. 1550 m, 11 Oct. 2014 leg. O. Ďuriška (SLO 1669). – Vikartovce, direction of Liptovská Teplička, submontane meadow, 2 Aug. 2014 leg. I. Vašičková (BRNM 781380). – Veporské vrchy Mts., Hriňová, Biele Vody, alt. 900–1000 m, 26 Sept. 2009 leg. O. Jindřich & J. Burel (Antonín 09.290, BRNM

781386). – Čierna hora Mts., Sedlice, alt. 500–700 m, 15 July 2008 leg. P. Kešeľák (SLO 1585). – Snina, Hrb hill, alt. 260 m, 21 May 2015 leg. J. Pavlík (BRNM 781410).

Slovenia. Kobatid, Livek, on pasture, alt. 800 m, 3 Oct. 2007 leg. R. Fontenla & R. Para (herb. R.P.–R.F. 071003-03). – Tolmin, Tolminski Lom, on meadow, alt. 610 m, 10 May 2004 leg. Z. Kavcic (herb. R.P.–R.F. 040510-01).

Melanoleuca cinereifolia

France. Vendée (85), Jard-sur-Mer, 11 Nov. 1988 leg. et det. R. Courtecuisse RC/F88135 (LIP, originally as *M. strictipes* fo.).

***Melanoleuca* sp. (subgen. *Macrocystis*)**

Belgium. Tervueren, 20 Sept. 1992 leg. Maerts, det. M. Bon 92209 (LIP, as *M. nivea* fo.).

TYPE STUDIES OF *MELANOLEUCA CINEREIFOLIA* (BON) BON

Melanoleuca strictipes* var. *cinereifolia Bon, Bulletin de la Société Mycologique de France 86: 155, 1970

Original diagnosis. A typo differt pileo maximo marginaque lobata, lamellis cinerascentibus et habitatione cam Psamma arenaria in thiniis littoralibus, paulo supra limitem superiorem plaxus. Ers. in herbario nostro (No. 91161 prope Le Crotoy et Fort-Mahon Somme) lectum. Lille 11-12-1969.

Holotype. France, Fort Mahon (Somme), pure dunes (Ammophiletum), 11 Dec. 1969 leg. M. Bon (LIP 91101; revised 21 July 2005).

Type revision. Spores 7.8–9.6 × 5.4–6.2 µm, average 8.54 × 5.82 µm, E = 1.34–1.61, Q = 1.47, shortly ellipsoid to ellipsoid, with hardly visible warts because weakly amyloid, however apparently isolated and rounded. Cheilocystidia very rare, narrowly lageniform (conform to those drawn by Bon in the form attached to the original material). Stipitipellis with paracystidia. Caulocystidia not seen. Pileipellis not seen because the specimen was very small.

Melanoleuca cinereifolia* var. *maritima Huijsman ex Bon, Documents Mycologiques 16(61): 46, 1985

Original diagnosis. A typo differt lamellis primo albidis, pileo vix obscuriore (Expo A-90 usque C-70), stipite vix breviore, cheilocystidiisque brevioribus vel compactis usque 50(65) × 15–20 µm. Habitatio eadem praecipue in Cakileto vel Agropyreto junceiformis.

Holotype. France, Somme, Cayeux, Le Hourdel, 18 Nov. 1976 (LIP, herbarium M. Bon 761118; revised 22 July 2005).

Type revision. Spores 7.2–10.8 × 4.8–6.2 µm, average 9.15 × 5.75 µm, E = 1.41–1.78, Q = 1.59, shortly ellipsoid to ellipsoid, with very small, isolated, rounded or elongated warts. Cheilocystidia frequent, lageniform but also ventricose. Stipitipellis made up of numerous tufts of clavate paracystidia, caulocystidia not seen.

Discussion

Melanoleuca strictipes is characterised by rather robust basidiomata, a glabrous, centrally brown (sometimes ochraceous brown) and marginally leathery yellow or almost cream pileus, cream coloured lamellae, a white or whitish stipe, whitish context, basidiospores with minute verruculose ornamentation, fusoid or sublageniform, macrocystidioid cheilo- and pleurocystidia, and caulocystidia of two types. It belongs to sect. *Alboflavidae* Singer.

In Europe, two other whitish coloured species of *Melanoleuca* occur, but both of them have urticoid cheilocystidia. One, *M. verrucipes* (Fr.) Singer, is easily distinguishable from *M. strictipes* by its unique black dots or scales on the stipe. The other one, *M. diverticulata* G. Moreno & Bon, may occasionally have an albino form, and may be mistaken for *M. strictipes*, but can be identified based on the cystidia.

When we compare our phylogeny results with Vizzini et al. (2011), their tree topology is in agreement with ours. The difference between their sequences of *M. strictipes* (JN616464–JN616466) and the newly obtained ones are probably caused by the bad analysis of raw sequence data before submission to GenBank.

Some authors distinguish *M. subalpina* from *M. strictipes*. According to Vesterholt (2012), *M. subalpina* is characterised by a shorter stipe than the pileus diameter, cheilocystidia with a rounded apex, and grows in dry grassland, pastures and alpine heathland. *Melanoleuca strictipes* is characterised by a longer stipe than the pileus diameter, cheilocystidia mostly with an acute apex, and grows in grassland, deciduous and mixed forests and in gardens. Bon (1991) considered *M. subalpina* a montane species with a white pileus and ochraceous yellowish centre. On the other hand, his concept of *M. strictipes* is different from other authors. The species has an initially pale beige or whitish, later grey-beige coloured pileus with a brown-olivaceous centre and a long-lasting paler, ± pruinose margin, and occurs in mossy or grassy deciduous forest habitats. Moser (1978) distinguished both species according to the presence of a farinaceous smell and a stipe longer than the pileus diameter in *M. strictipes*. However, the sequences of the epitype of *M. subalpina* match with those of *M. strictipes*.

Kühner (1978) described a new species, *Melanoleuca substrictipes*, based on material from Savoie, France. The holotype specimen consists of two basidiomata representing quite different species (even from different subgenera) – part A represents *M. exscissa* (GenBank accession no. LT594134; Antonín et al. in preparation), and part B a macrocystidioid *M. strictipes*.

Some authors distinguish another white species, *M. nivea* Métrod ex Boekhout, in sect. *Alboflavidae*. It differs by smaller basidiomata (pileus 30–50 mm broad, stipe 30–55 × 4–7.5 mm) with a grey-brown stipe finally becoming greyish in the upper part and brownish towards the base, context whitish in pileus, brown to

orange-brown in the upper stipe part and dark brown towards the base, and smaller basidiospores [7–8.5(9) × 4–5 µm] and cheilo- and pleurocystidia [(35)40–65 × 9–15(20) µm]. It grows in grassland on coastal dunes and on sandy soils (Boekhout 1988, 1999, Bon 1991, Watling & Turnbull 1998, Gröger 2006). According to the description, it should be a fungus different from *M. strictipes*. However, Vizzini et al. (2011) studied several collections of *M. nivea*, whose sequences belonged to three separate clades outside sect. *Alboflavidae*. According to these authors, none of them represented *M. nivea* – they were (1) an albino form of *M. melaleuca* s. Fontenla et al. (2003), (2) a taxon close to *M. robusta* (Bres.) Fontenla, Gottardi & Para [= *Melanoleuca humilis* var. *robusta* (Bres.) Bon], and (3) probably an albino form of *M. albifolia* Boekhout. We received the ITS sequence of a specimen of *M. nivea* identified by Bon, which seems to be a separate lineage. It is necessary to study new collections of it and select an epitype.

Velenovský (1920) described another white-coloured macrocystidioid species, *Tricholoma candidum* Velen. [= *Melanoleuca candida* (Velen.) Singer] with a 60–100 mm broad, pure white (pale ochraceous when moist) pileus, a white stipe about as long as the pileus diameter, and white, pinkish tinged lamellae. Type revision confirmed that it belongs to *M. strictipes*. However, the name *T. candidum* (and the subsequent combination) is invalid because of the presence of the older name *Tricholoma candidum* A. Blytt 1905. The sequence JN052142 generated by Vizzini et al. (2011) was identified as *M. evenosa* (herbarium spec. MCVE 14576) in the paper but this is labelled as *M. candida* in GenBank. According to Bon (1991), *Melanoleuca parisianorum* R. Haller Aar. ex Bon (with var. *clitocyboides* Bon) should be close to *M. nivea* and *M. subalpina*, the main differential character being the presence of a distinct, aniseed-, violet- or *Hebeloma sacchariolens*-like smell. We sequenced the type specimen of var. *clitocyboides* (Italy, Val de Rabbi, 1 Sept. 1983, leg. Gruppo Micologico Bresadola, LIP, herb. Bon 83102), but the sequencing failed.

Melanoleuca cinereifolia and its var. *maritima* differ strongly from *M. strictipes* in having the following characters: pileus dark greyish-brown, stipe more or less concolorous, lamellae greyish, and context in the lower part of the stipe darker brownish. Furthermore, it grows on sandy sea dunes. *Melanoleuca cinereifolia* has not been recorded in the Czech Republic or Slovakia.

The ecological amplitude of the studied collections of *M. strictipes* is rather wide. The species grows in lowland deciduous forests (BRNM 751690), upland grasslands (SLO 1661), on mountain pastures (SLO 1669) up to alpine meadows and snow-patch grasslands (BRNM 781383 and BRNM 781303). The altitude range of the localities is 190–2280 m. The ecology encompasses that of both species, *M. strictipes* and *M. subalpina*, in the literature (see above). Therefore, the range of habitats also supports the conspecificity of both taxa.

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