ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/118.361

Volume 118, pp. 361–381 *October–December 2011*

A preliminary ITS phylogeny of *Melanoleuca* (*Agaricales*), with special reference to European taxa

Alfredo Vizzini[1*], Roberto Para[2], Roberto Fontenla[3], Stefano Ghignone[4], Enrico Ercole[1]

[1]*Dipartimento di Biologia Vegetale - Università degli Studi di Torino Viale Mattioli 25, I-10125, Torino, Italy*

[2] *Via Martiri di Via Fani 22, I-61024, Mombaroccio (PU), Italy*

[3] *Via Monte Marino 26, I-60125 Ancona, Italy*

[4] *Istituto per la Protezione delle Piante, CNR Sezione di Torino, Viale Mattioli 25, I-10125, Torino, Italy*

Correspondence to *: *alfredo.vizzini@unito.it*

Abstract — Ninety-one *Melanoleuca* collections were chosen to test the agreement of current and traditional infrageneric tripartite classifications of *Melanoleuca* (subgenera *Acystis* without cystidia, *Urticocystis* with urticiform cystidia, and *Melanoleuca* with macrocystidia) with molecular phylogenetic data (ITS sequences analysis) and to evaluate the systematic significance of relevant morphological characters. *Melanoleuca* is found to be monophyletic, and only two emended subgenera, *Urticocystis* and *Melanoleuca*, are supported. Subg. *Urticocystis* comprises all taxa with urticoid cystidia plus the macrocystidiate *M. cognata* complex. The artificial subg. *Acystis* is shown to be polyphyletic and no longer tenable. The entire genus comprises at least 10 clades with 13 subclades. *Melanoleuca sublanipes* sp. nov. and new combinations *M. exscissa* f. *iris*, *M. exscissa* f. *sarcophylla*, *M. exscissa* f. *diverticulata*, and *M. paedida* f. *electropoda* are introduced.

Key words — *Basidiomycota*, *Agaricomycetes*, *Kinia*, pluteoid clade, taxonomy

Introduction

Kirk et al. (2008) state that the basidiomycete genus *Melanoleuca* Pat., typified by *M. vulgaris* (Pat.) Pat. [= *M. melaleuca* (Pers.) Murrill] and traditionally placed in subtribe *Leucopaxillineae* Singer (*Tricholomataceae* R. Heim ex Pouzar, *Agaricales* Underw.) (Singer 1986), comprises approximately 50 saprobic species worldwide. However, Index Fungorum (http://www.indexfungorum.org/, accessed 28 June 2011), lists 332 validly published *Melanoleuca* names representing 126 European and 206 extra-European taxa (pers. obs.). Of the 164 names that actually represent the genus as presently circumscribed (cf. Pfister

1984), many are now known as synonyms with others yet to be listed as such. New taxa are, however, continuously being described, even from well-studied areas such as Europe (e.g., Bresinsky 2006, this paper). *Melanoleuca* species are cosmopolitan and characterised by the following characters: collybioid to tricholomatoid basidiomata; convex to slightly depressed (often with a shallow umbo) pilei; emarginated to adnate to shortly decurrent lamellae; absence of veils; white to pale-yellowish spore print; cutis to trichoderm pileipellis; hyaline spores with amyloid ornamentations; cheilocystidia (mostly present) of two types either urticoid, septate, thin-walled or fusiform to lageniform, mostly aseptate, slightly thick-walled, sometimes with encrusting crystals at apex; pleurocystidia similar to cheilocystidia; no clamp connections (Pegler & Young 1973, Gillman & Miller 1977, Kühner 1978, Singer 1986, Boekhout 1988, 1999; Bon 1991, Vesterholt 2008, Watling & Turnbull 2008). *Leucopaxillus* Boursier, a morphologically allied genus, differs from *Melanoleuca* mainly in abundant clamp connections and (usually) lacking well-developed hymenial cystidia (Singer 1986, Bon 1991). But, according to recent molecular analyses (Moncalvo et al. 2002, Matheny et al. 2006), *Melanoleuca* and *Leucopaxillus* are not closely related: *Melanoleuca* species cluster within the Pluteoid clade (*Pluteaceae* Kotl. & Pouzar), whereas *Leucopaxillus* belongs to the Tricholomatoid clade, close to *Tricholoma* (Fr.) Staude. Sequence analyses by Moncalvo et al. (2000, 2002) place *Melanoleuca* and *Pluteus* Fr. as sister to *Amanitaceae* R. Heim ex Pouzar, while others place the minute uniloculate gasteromycete, *Limnoperdon incarnatum* G.A. Escobar, sister to *Melanoleuca* and *Pluteus* (Bodensteiner et al. 2004; Binder et al. 2006; Vizzini et al. 2010) or to *Melanoleuca*, *Pluteus* and *Volvariella* Speg. (Matheny et al. 2006). Justo et al. (2011) recently place *Melanoleuca* as sister to a monophyletic group formed by *Pluteus* species and *Volvopluteus* Vizzini et al. Finally, Vizzini et al. (2010) reduced the puzzling genus *Kinia* Consiglio et al. to a subgenus of *Melanoleuca* with non-amyloid spores.

Melanoleuca is one of the less appealing fungal genera, whose members are mostly tedious and drab in appearance and dull in pileus colours. It is a character-poor genus with many species macroscopically very similar and differing only in very subtle features (e.g., basidioma colour, odour, stipe ornamentation) and a morphology strongly influenced by environmental factors (Bon 1991, Boekhout 1999). Thus far, infrageneric classifications and species circumscriptions have relied on morphological characters. Identification especially depends on microscopic observations of a rather limited set of characters, such as presence/absence of cheilocystidia, cystidial shape, spore size and ornamentation, and pileipellis structure. Interpretation of some characters may rely on personal experience (e.g. shape of cystidia); in some cases the value ranges overlap (e.g. spore size), in others pileipellis structure and spore

size show great intra-basidiome variability. From a traditional morphological perspective, this often makes species identification difficult or even daunting. The paucity of *Melanoleuca* characters has been detrimental to establishing a natural taxonomic framework for rationalizing infrageneric relationships.

The lack of a modern *Melanoleuca* monograph and the existence of many short, taxonomically and geographically limited publications further complicate the situation. In addition, there are many controversies concerning the interpretations of old descriptions and names (Boekhout 1988).

Several different infrageneric classifications of *Melanoleuca* have been published. Singer (1935, 1943, 1986), Métrod (1942, 1948), Kühner (1978), and Moser (1983) proposed schemes based mainly on macromorphological features (see Boekhout 1988 for an historical review). Singer (1986), for example, divided *Melanoleuca* into four sections —*Alboflavidae* Singer, *Humiles* Singer, *Oreinae* Singer, *Melanoleuca*— circumscribed only by pileus colour and stipe ornamentation.

Bon (1978), the first to present an infrageneric classification based equally on macro- and micro-morphological characters, divided *Melanoleuca* into seven sections. Boekhout (1988), focusing mainly on microscopic features, stressed the importance of absence/presence and shape of cystidia (first noted by Metrod 1948) and divided the genus into three subgenera (Tab. 1): subg. *Melanoleuca*—no cystidia; subg. *Urticocystis* Boekhout—with urticiform cheilocystidia and no (or very rare) pleurocystidia; subg. *Macrocystis* Boekhout—with long fusiform to lageniform cheilocystidia (macrocystidia) and similar pleurocystidia. Boekhout recognized two urticiform types: the *brevipes*-type (with narrow cylindrical upper part, Fig. 1a) and the *exscissa*-type (with rather wide upper part attenuating towards the apex, Fig. 1b).

Bon (1991) proposed a slightly different infrageneric classification (Tab. 1) by introducing the spore Q value (the ratio of length to width of the spores in side view) and stressing the importance of fusiform versus lageniform cystidia (Fig. 1c–d) as important key characters for delimiting subsections.

Finally, Boekhout (1999) reintroduced a simplified classification practically identical to the 1988 scheme except that sect. *Grammopodiae* was no longer subdivided into subsects. *Grammopodiae* and *Exscissae* (Tab. 1).

There are only two major monographic treatments of *Melanoleuca*: Bon (1991) covers over 80 taxa, most reported only from Europe, and Boekhout (1999), covers only Dutch taxa and unites species into large complexes thereby recognizing only 14 species and several forms and varieties.

Bon (1991), who covered a larger number of taxa, was used both for selecting which species to sample and for testing the effectiveness of Bon's classification method. Due to the limitation of morphological characters used, several of his taxonomical units remain controversial.

Table 1. The tripartite infrageneric classifications of *Melanoleuca* by Bon and Boekhout.

Boekhout's classification (1988, 1999)
Subg. *Melanoleuca* – without cystidia.
Subg. *Urticocystis* – cheilocystidia urticiform and pleurocystidia absent or very rare.
Sect. *Humiles* – stipe squamulose or verrucose throughout; stipe usually much longer than diameter of the pileus.
Sect. *Grammopodiae* – stipe smooth or somewhat fibrillose; stipe much shorter than or equally large as diameter of pileus.
*Subsect. *Grammopodiae* – urticiform cystidia of the *brevipes*-type.
*Subsect. *Exscissae* – urticiform cystidia of the *exscissa*-type.
Subg. *Macrocystis* – cheilocystidia fusiform to lageniform and pleurocystidia similar.
Sect. *Cognatae* – with a bright coloured pileus.
Sect. *Alboflavidae* – with a white to cream-whitish pileus.
Sect. *Strictipedes* – with a grey-brown pileus.
Bon' s classification (1991)
Subg. *Acystis* – species without cystidia.
Sect. *Acystis* – spores with a Q<1.4(–1.5).
Sect. *Decembres* – spores with a Q>(1.5–) 1.6.
Subg. *Urticocystis* – cheilocystidia urticiform to strictly lageniform, septate, up to 50(–60) µm long.
Sect. *Humiles* – stipe squamulose to dark-dotted.
Sect. *Grammopodiae* – stipe smooth or striate, sometimes pruinose or flocky.
Subsect. *Rasilinae* – spores with a Q<1.4(–1.5), with isolated warts, cystidia typically urticiform.
Subsect. *Grammopodiae* – spores with a Q>(1.5–)1.6, cystidia typically urticiform.
Subsect. *Exscissae* – spores with a Q>(1.5–)1.6, urticiform cystidia of the *exscissa*-type.
Subg. *Melanoleuca* – macrocystidia fusiform to lageniform, non-septate, up to (40–)50–90(–110) µm long.
Sect. *Alboflavidae* – basidiomes white to whitish.
Sect. *Cognatae* – basidiomes with bright colours, pileus and lamellae concolorous.
Sect. *Oreinae* – basidiomes small (<4(–6) cm), collybioid, often dark coloured.
Sect. *Melanoleuca* – basidiomes small to medium-sized (>(5–) 6 cm), with variable colourations.
Subsect. *Strictipedinae* – cystidia mainly lageniform.
Subsect. *Vulgarinae* – cystidia mainly fusiform.

* = subsections present in the 1988 classification scheme only.

The present study is based on a large ITS sequence dataset and is the first to examine *Melanoleuca* extensively. Our aims were to 1) check whether *Melanoleuca* is monophyletic as traditionally circumscribed; 2) test Bon's (1991) morphologically based taxonomy against molecular phylogenetic data; 3) evaluate whether traditional or other morphological features (e.g., pileus colour, spore and cheilocystidia shape) reflect phylogenetic relationships.

Materials & methods

Taxon sampling

Samples from 91 *Melanoleuca* collections (41 taxa, 18 unidentified *Melanoleuca* sp.; Tab. 2) were tested. Specimens were collected fresh, dried, and deposited in ANC (Erbario

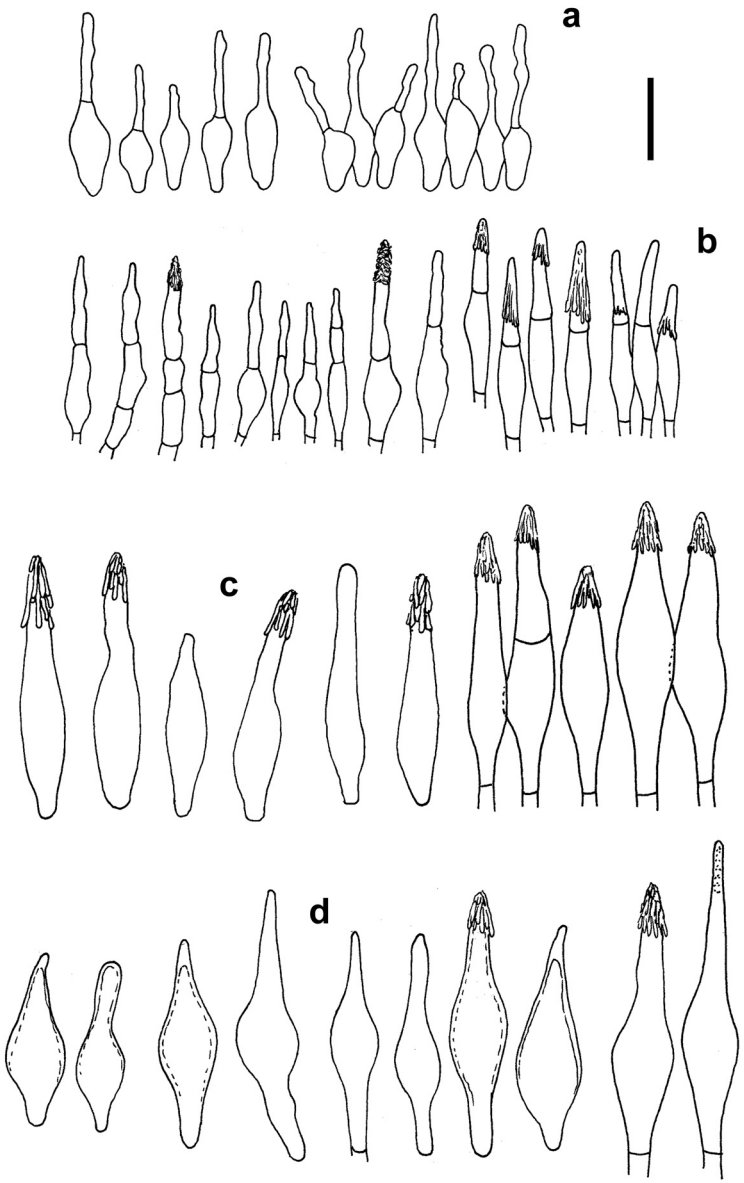

Figure 1. Types of cystidia in *Melanoleuca*.
a–b. Urticiform cystidia (a = *brevipes*-type; b = *exscissa*-type).
c–d. Macrocystidia (c = fusiform; d = lageniform).
Bar = 20 µm.

Table 2. *Melanoleuca* collections examined (species with multiple collections numbered consecutively; see also Fig. 2).

Collections	Coll. ID./Origin	Subgenera / Sections (Bon 1991)	ITS Acc. No.
* *Melanoleuca albifolia* 1	ANC M0182/Spain	*Melanoleuca / Oreinae*	JN616418
* *M. albifolia* 2	ANC M0184/Italy	*Melanoleuca / Oreinae*	JN616419
* *M. angelesiana* 1	ANC M0203/Italy	*Acystis / Acystis*	JN616420
* *M. angelesiana* 2	ANC M0204/Italy	*Acystis / Acystis*	JN616421
* *M. arcuata*	ANC M0167/Italy	*Melanoleuca / Cognatae*	JN616422
* *M. atripes*	ANC M0180/Italy	*Melanoleuca / Oreinae*	JN616423
* *M. bataillei*	ANC M0185/Italy	*Melanoleuca / Melanoleuca*	JN616424
M. brevipes 1	MCVE 04574/Italy	*Urticocystis /Grammopodiae*	JF908352
M. brevipes 2	MCVE 04505/Italy	*Urticocystis /Grammopodiae*	JF908351
M. cinereifolia 1	MCVE 01471/Italy	*Melanoleuca / Melanoleuca*	JN052138
M. cinereifolia 2	MCVE 11243/Italy	*Melanoleuca / Melanoleuca*	JF908356
M. cinereifolia 3	MCVE 20748/Italy	*Melanoleuca / Melanoleuca*	JN052137
M. cognata 1	MCVE 13939/Italy	*Melanoleuca / Cognatae*	JF908360
* *M. cognata* 2	ANC M0170/Italy	*Melanoleuca / Cognatae*	JN616425
* *M. decembris* 1	ANC M0197/Italy	*Acystis / Decembres*	JN616426
* *M. decembris* 2	ANC M0199/Italy	*Acystis / Decembres*	JN616427
* *M. decembris* 3	ANC M0200/Italy	*Acystis / Decembres*	JN616428
M. decembris 4	MCVE 01573/Italy	*Acystis / Decembres*	JF908346
* *M. diverticulata*	ANC M0206/Italy	*Urticocystis /Grammopodiae*	JN616429
* *M. electropoda*	ANC M0187/Italy	*Melanoleuca / Oreinae*	JN616430
M. evenosa	MCVE 14576/Italy	*Melanoleuca / Alboflavidae*	JN052142
* *M. exscissa* 1	ANC M0207/Italy	*Urticocystis /Grammopodiae*	JN616431
* *M. exscissa* 2	ANC M0208/Italy	*Urticocystis /Grammopodiae*	JN616432
* *M. exscissa* 3	ANC M0210/Italy	*Urticocystis /Grammopodiae*	JN616433
* *M. exscissa* 4	ANC M0210B/Italy	*Urticocystis /Grammopodiae*	JN616434
* *M. exscissa* 5	ANC M0212/Italy	*Urticocystis /Grammopodiae*	JN616435
* *M. exscissa* 6	ANC M0213/Italy	*Urticocystis /Grammopodiae*	JN616436
* *M. friesii*	ANC M0186/Italy	*Melanoleuca / Melanoleuca*	JN616437
* *M. graminicola*	ANC M0201/Italy	*Acystis / Acystis*	JN616438
* *M. grammopodia* 1	ANC M0217/Italy	*Urticocystis /Grammopodiae*	JN616439
* *M. grammopodia* 2	ANC M0218/Italy	*Urticocystis /Grammopodiae*	JN616440
* *M. grammopodia* 3	ANC M0219/Slovenia	*Urticocystis /Grammopodiae*	JN616441
* *M. grammopodia* f. *macrocarpa* 1	ANC M0215/Italy	*Urticocystis /Grammopodiae*	JN616442
* *M. grammopodia* f. *macrocarpa* 2	ANC M0216/Italy	*Urticocystis /Grammopodiae*	JN616443
M. grammopodia f. *macrocarpa* 3	MCVE 04410/Italy	*Urticocystis /Grammopodiae*	JF908350
* *M. heterocystidiosa* 1	ANC M0174/Italy	*Melanoleuca / Melanoleuca*	JN616444
* *M. heterocystidiosa* 2	ANC M0175/Italy	*Melanoleuca / Melanoleuca*	JN616445
* *M. iris*	ANC M0211/Italy	*Urticocystis /Grammopodiae*	JN616446
* *M. "lanipes"*	ANC M0166/Italy	*Melanoleuca / Melanoleuca*	JN616447
* *M. melaleuca*	ANC M0176/Italy	*Melanoleuca / Melanoleuca*	JN616448
* *M. microcephala*	ANC M0196/Italy	*Urticocystis /Grammopodiae*	JN616449
* *M. nivea* 1	ANC M0177/Italy	*Melanoleuca / Alboflavidae*	JN616450
* *M. nivea* 2	ANC M0183/Italy	*Melanoleuca / Alboflavidae*	JN616451
M. nivea 3	MCVE 09578/Italy	*Melanoleuca / Alboflavidae*	JN392452

* = collections newly sequenced in this study.

Table 2, concluded

Collections	Coll. ID./Origin	Subgenera / Sections (Bon 1991)	ITS Acc. No.
M. oreina	MCVE 07839/Italy	*Melanoleuca / Oreinae*	JN392450
* *M. paedida* 1	ANC M0189/Italy	*Urticocystis /Grammopodiae*	JN616452
* *M. paedida* 2	ANC M0190/Italy	*Urticocystis /Grammopodiae*	JN616453
M. "paratristis"	MCVE 12645/Italy	*Acystis / Acystis*	JF908357
* *M. privernensis*	GC 08310 holotype/Italy	Subgen. *Kinia* (Vizzini et al. 2010)	JN616454
* *M. pseudoluscina* 1	ANC M0191/Italy	*Urticocystis /Grammopodiae*	JN616455
* *M. pseudoluscina* 2	ANC M0192/Italy	*Urticocystis /Grammopodiae*	JN616456
* *M. pseudoluscina* 3	ANC M0193/Italy	*Urticocystis /Grammopodiae*	JN616457
* *M. pseudoluscina* 4	ANC M0194/Italy	*Urticocystis /Grammopodiae*	JN616458
* *M. pseudoluscina* 5	ANC M0195/Italy	*Urticocystis /Grammopodiae*	JN616459
* *M. pseudopaedida*	ANC M0198/Italy	*Acystis / Acystis*	JN616460
* *M. rasilis*	ANC M0220/Italy	*Urticocystis /Grammopodiae*	JN616461
* *M. robertiana*	ANC M0205/Italy	*Acystis / Acystis*	JN616462
* *M. robusta*	ANC M0179/Italy	*Melanoleuca / Melanoleuca*	JN616463
* *M. strictipes* 1	ANC M0171/Italy	*Melanoleuca / Alboflavidae*	JN616464
* *M. strictipes* 2	ANC M0172/Italy	*Melanoleuca / Alboflavidae*	JN616465
* *M. strictipes* 3	ANC M0173/Italy	*Melanoleuca / Alboflavidae*	JN616466
* *M. stridula*	ANC M0007/Italy	*Acystis / Acystis*	JN616467
* *M. striimarginata*	ANC M0202/Italy	*Acystis / Acystis*	JN616468
M. subalpina	MCVE 04112/Italy	*Melanoleuca / Alboflavidae*	JN052139
* *M. sublanipes* 1	ANC M0221/Italy	*Urticocystis /Grammopodiae*	JN616469
* *M. sublanipes* 2	ANC M0222 holotype/Italy	*Urticocystis /Grammopodiae*	JN616470
* *M. sublanipes* 3	ANC M0223/France	*Urticocystis /Grammopodiae*	JN616471
* *M. subpulverulenta* 1	ANC M0004/Italy	*Melanoleuca / Melanoleuca*	JN616472
* *M. subpulverulenta* 2	ANC M0178/Italy	*Melanoleuca / Melanoleuca*	JN616473
* *M. substrictipes* 1	ANC M0214/Italy	*Urticocystis /Grammopodiae*	JN616474
M. substrictipes 2	MCVE 13934/Italy	*Urticocystis /Grammopodiae*	JF908359
* *M. substrictipes* var. *sarcophylla*	ANC M0209/Italy	*Urticocystis /Grammopodiae*	JN616475
M. verrucipes	MCVE 09962Switzerland	*Urticocystis / Humiles*	JF908354
* *Melanoleuca* sp. 1	ANC M0181/Italy	*Acystis / Acystis*	JN616476
* *M.* sp. 2	ANC M0188/Italy	*Acystis / Acystis*	JN616477
* *M.* sp. 3	ANC M0224/Italy	*Melanoleuca / Melanoleuca*	JN616478
M. sp. 4	MCVE 12248/Italy	*Melanoleuca / Melanoleuca*	JN052141
M. sp. 5	MCVE 13410/Italy	*Melanoleuca / Melanoleuca*	JF908358
M. sp. 6	MCVE 09821/Italy	*Melanoleuca / Melanoleuca*	JN392453
M. sp. 7	MCVE 01687/Italy	*Melanoleuca / Melanoleuca*	JN392449
M. sp. 8	MCVE 01683/Italy	*Melanoleuca / Melanoleuca*	JN392448
M. sp. 9	MCVE 19223/Italy	*Melanoleuca / Melanoleuca*	JN392446
M. sp. 10	MCVE 08389/Italy	*Melanoleuca / Cognatae*	JF908353
M. sp. 11	MCVE 08432/Italy	*Melanoleuca / Melanoleuca*	JN052140
M. sp. 12	MCVE 14221/Italy	*Urticocystis /Grammopodiae*	JN392454
M. sp. 13	MCVE 08384/Italy	*Urticocystis /Grammopodiae*	JN392451
M. sp. 14	MCVE 03316/Italy	*Urticocystis /Grammopodiae*	JF908349
M. sp. 15	MCVE 19627/Italy	*Urticocystis /Grammopodiae*	JF908344
* *M.* sp. 16	MCVE 24095/Italy	*Urticocystis /Grammopodiae*	JN616479
M. sp. 17	MCVE 01681/Italy	*Urticocystis /Grammopodiae*	JN392447
M. sp. 18	MCVE 14273/Italy	*Acystis / Decembres*	JF908362

dell'Università di Ancona, Italy) or acquired from MCVE (the Museo di Storia Naturale di Venezia Herbarium, Italy) (Tab. 2). MCVE collections were chosen, even where often misdetermined, because DNA had already been bar-coded by Dr. M. Garbelotto (University of California, Berkeley). All *Melanoleuca* collections were identified or redetermined using i) an unpublished key based on the protologues or referenced to original collections (Fontenla & Para, ined.) and ii) existing monographs (Bon 1991, Boekhout 1999, Fontenla et al. 2003). Watling & Turnbull (1983), Horak (2005), and Vesterholt (2008) were also consulted. When not identifiable, collections are cited in Tab. 2 and Fig. 2 as *Melanoleuca* sp. *Melanoleuca* species were selected to represent subgenera and sections in Bon (1991; Tab. 1). Each section was represented by at least two species except for sect. *Humiles* (represented only by *M. verrucipes*). *Melanoleuca privernensis* (Consiglio et al.) Consiglio et al. [≡ *Kinia privernensis*] was also included in the dataset in accordance with Vizzini et al. (2010). In the species descriptions Q = quotient of length and width of the spores in side view and Qm = average quotient. The spore Qm value and cystidia shape are reported for each collection in Fig. 2. Herbarium abbreviations follow Thiers (2011). Author citations follow the Index Fungorum-Authors of Fungal Names (http://www.indexfungorum.org/authorsoffungalnames.htm) and the names of new taxa are deposited in MycoBank (http://www.mycobank.org/DefaultPage.aspx).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was isolated, extracted from 62 dried herbarium specimens (Tab. 2) using the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy). Universal primers ITS1F/ITS4 were used for ITS region amplification (White et al. 1990, Gardes & Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in 25 mL reaction mixtures with these final concentrations or total amounts: 5 ng DNA, 13 PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 mM each primer, 2.5mM MgCl2, 0.25mM each dNTP, 0.5 unit Taq polymerase (Promega). The PCR program was 3 min at 95 C for one cycle, 30 s at 94 C, 45 s at 50 C, 2 min at 72 C for 35 cycles, 10 min at 72 C for one cycle. PCR products were resolved on a 1.0%agarose gel and visualized by staining with ethidium bromide. PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy). Sequences were assembled and edited with the phred/phrap/consed software suite and submitted to GenBank (Tab. 2).

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were generated in this study (Tab. 2) or retrieved from GenBank. GenBank sequences were selected based on other phylogenetic studies on *Agaricales* (Moncalvo et al. 2002; Matheny et al. 2006; Justo et al. 2011). *Limnoperdon incarnatum* (DQ097363) was used as outgroup taxon. Other taxa belonging to the Pluteoid clade were included in the analysis for testing the monophyly of *Melanoleuca*.

Sequences obtained in this study were checked and assembled using Geneious v5.3 (Drummond et al. 2010). Alignment of the ITS dataset was generated using MAFFT v6.814b (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The alignment was then imported into MEGA 5.0 (Tamura et al. 2011) for

manual adjustment. Best-fit models were estimated by both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) using jModelTest 0.1.1 (Posada 2008) to provide a substitution model for each single alignment. TPM2uf+I+G model was chosen. Phylogenetic hypotheses were constructed under Bayesian Inference (BI) and Maximum Likelihood (ML) criteria.

BI of phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 10.000.000 generations, under model assumption. Trees were sampled every 1.000 generations resulting in an overall sampling of 10.001 trees. The "burn-in" value was evaluated using Tracer 1.5 (Rambaut & Drummond 2007). The first 15% of trees was discarded as "burn-in". For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only BPP values over 50% are reported in the resulting trees. This Bayesian analysis was repeated three times, always using random starting trees and random starting values for model parameters to test the independence of the results from the revisiting of the prior topologies during chain growth (Huelsenbeck et al. 2002).

ML estimation was performed through RAxML v.7.0.4 (Stamatakis 2006) with 1,000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMAI algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. Support values for major clades that are supported by either BI and ML analyses are visualized in the resulting tree.

Analysis of the pairwise % identity values (hereafter shortened as P%IV) for the *Melanoleuca* sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

Results

Our phylogenetic results are presented in Fig. 2. The general ITS data matrix comprises a total of 105 sequences (including 14 from GenBank). This 898 bp dataset contains 621 (69.2%) variable sites, of which 525 (58.4%) are parsimony-informative. The Bayesian and ML tree topologies are congruent. Our analyses show that *Melanoleuca* is clearly monophyletic (1.0 BPP and 89% MLB). Two major clades, A and B, were distinguished within *Melanoleuca*. Clade A is supported by only BI tree with 0.79 BPP, while clade B is well supported with 1.0 BPP and 99% MLB. Clade A consists of 5 clades (A1–A5, with 10 subclades) and clade B of 5 clades (B1–B5, with 3 subclades).

Discussion

Melanoleuca and infrageneric classification

The Bayesian (1.0 BPP) and ML (89% MLB) analyses strongly support *Melanoleuca* as monophyletic and distributed throughout the ingroup within

2 major clades (A and B) with 10 smaller clades (A1–A5 and B1–B5) (Fig. 2). The two sequences of *M. subsejuncta* (Peck) Murrill from GenBank (FJ596898; FJ596898) cluster outside *Melanoleuca*, sister to *Amanitaceae*; Pfister (1984), who examined its type, referred the collection to *Tricholoma*. Results of our phylogenetic analyses and the current morphology-based infrageneric classification of *Melanoleuca* are not congruent. In particular, our data (Fig. 2) are incompatible with the tripartite infrageneric *Melanoleuca* classification of Bon (1991) and Boekhout (1988, 1999) (Tab. 1). Based on molecular data, species traditionally ascribed to subgenus *Acystis* Bon (= subgenus *Melanoleuca* sensu Boekhout 1988, 1999) do not form a monophyletic assemblage and are distributed over the *Melanoleuca* clade (clade A and B, Fig. 2).

The fact that acystidiate taxa are not phylogenetically related implies that cystidial acquisition and loss have taken place independently during the evolution of *Melanoleuca* and also that this character is homoplastic and unsuitable for a natural classification of these fungi. Subgenus *Acystis* should therefore be considered an artificial superfluous taxon that is no longer tenable. In addition, in some cases (e.g. subclades A4.1 & A4.2; clade B2, Fig. 2), cystidiate and non-cystidiate taxa are conspecific (syntaxic): the acystidiate *M.* sp. 18, *M.* sp. 2, and *M.* sp. 1 represent only forms of the cystidiate taxa *M. pseudoluscina* Bon, *M. paedida* (Fr.) Kühner & Maire, and *M. atripes* Boekhout/*M. albifolia* Boekhout, respectively. It is conceivable that the shift from cystidiate to non-cystidiate basidiomata and vice versa within a single species is controlled by a limited number of genes that are switched off by so far unknown environmental factors.

Most macrocystidiate taxa in subg. *Melanoleuca* (= *Macrocystis* Boekhout) form clade B (Fig. 2), while the *M. cognata* complex (A5 clade, = sect. *Cognatae*) nests with all species in subg. *Urticocystis* to form clade A (Fig. 2). Therefore, subg. *Melanoleuca* as traditionally circumscribed (Bon 1991) is polyphyletic and subg. *Urticocystis* is paraphyletic when sect. *Cognatae* is excluded. It would appear that even cystidial shape is not a reliable character for tracing higher phylogenetic relationships.

Our analysis therefore implies that only two subgenera (clades A and B) should be recognized in *Melanoleuca*:

Melanoleuca Pat. subg. ***Melanoleuca***, emend. Fontenla, Para & Vizzini

Clade B (the autonymous subgenus, including *M. melaleuca*), characterized by basidiomata with non-septate macrocystidia, or rarely without cystidia.

Melanoleuca subg. ***Urticocystis*** Boekhout, Persoonia 13(4): 400 (1988), emend. Fontenla, Para & Vizzini

Clade A (type species: *M. grammopodia* (Bull.) Murrill), comprising taxa mainly with urticocystidia but also with macrocystidia and brightly coloured pilei (sect. *Cognatae*), or lacking cystidia.

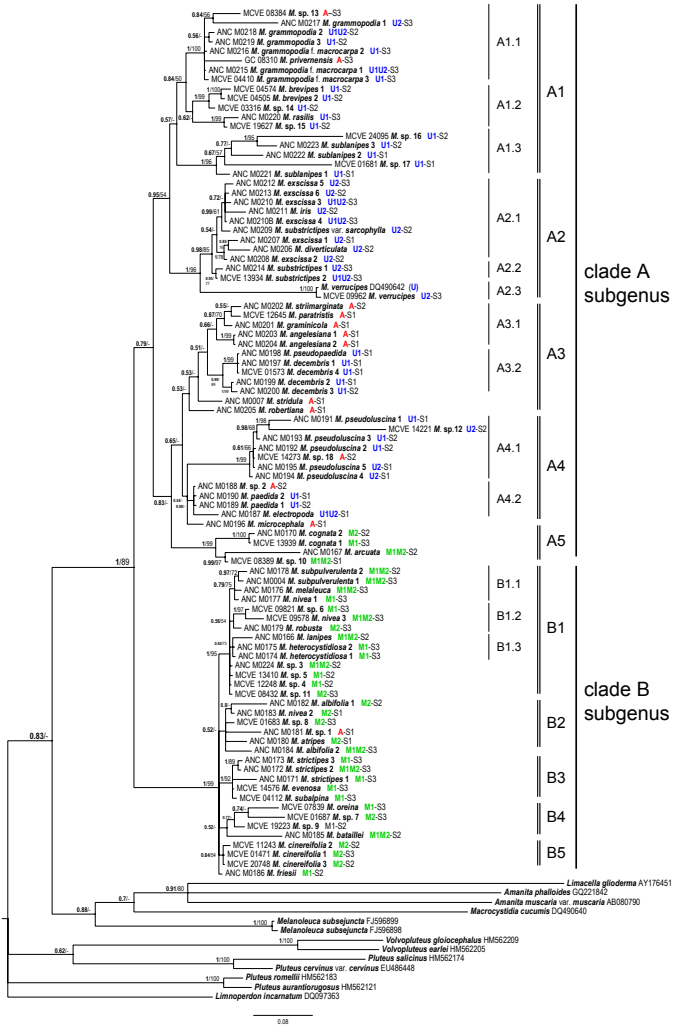

FIGURE 2. Bayesian phylogram obtained from the ITS rDNA sequences of *Melanoleuca* and other taxa of the Pluteoid clade. *Limnoperdon incarnatum* was used as outgroup taxon. Support values (**BPP** in bold >0.5; MLB >50%) are given above branches. Minor supported clades discussed in the text are numbered A1–A5 and B1–B5. Numbers refer to the collections cited in TAB. 2.

Characters: 1) presence/absence of cheilocystidia and type of cheilocystidia — **A** = taxa without cheilocystidia, **U1** = taxa with *brevipes*-type urticiform cystidia, **U2** = taxa with *exscissa*-type urticiform cystidia, **U1-U2** = taxa with both types of urticiform cystidia, **M1** = taxa with fusiform macrocystidia, **M2** = taxa with lageniform macrocystidia, **M1-M2** = taxa with both type of macrocystidia [cystidial types are coded in red (A), blue (U), and green (M)]; 2) spore shape (Qm range) – **S1** = Qm < 1.40; **S2** = 1.40≤ Qm ≤1.60; **S3** = Qm > 1.60..

Basidiospore shape (Qm value) seems to be homoplastic, too variable to use for circumscribing sections and subsections as proposed by Bon (1991). For example, in subclade A2.1, collections otherwise assignable to *M. exscissa* (Fr.) Singer show a great Qm range, as do *M. grammopodia* (A1.1) and *M. pseudoluscina* (A4.1) collections (Fig. 2).

On the contrary, cystidial subtypes may have some value for delimiting small clades (Fig. 2). Clades A1 and A3 are formed mainly by taxa with *brevipes*-type urticocystidia and clade A2 by taxa by mainly *exscissa*-type urticocystidia. Clade B1 comprises taxa with mainly fusiform macrocystidia.

Pileus colour is not a reliable phylogenetic marker at any taxonomic level: three collections (ANC M0177, MCVE 09578, ANC M0183) determined as *M. nivea* Métrod ex Boekhout, a species traditionally included in sect. *Alboflavidae* based on whitish colouration and presence of macrocystidia (Bon 1991, Boekhout 1988, 1999; Deschuyteneer 2008), are not placed with other species of that section (clade B3) but distributed throughout clade B. The first collection represents an albinic form of *M. melaleuca* (B1.1), the second a taxon close to *M. robusta* (Bres.) Fontenla et al. (B1.2), and the third probably an albinic form of *M. albifolia* (B2).

In conclusion, taxonomically important morphological characters in *Melanoleuca* show a high degree of homoplasy. Although these characters are useful for species delimitation, and in some cases for the circumscription of sections or groups, they appear insufficient for a phylogenetically correct infrageneric concept. A clearer picture will emerge as more *Melanoleuca* diversity is included in future analyses.

Groupings and species limits

More extensive sampling of *Melanoleuca* is needed for a revision of the whole genus. Additional ITS sequencing is necessary to clarify species limits and names for taxa occurring in both Europe and North America. For the time being we only make minor comments on some of the infrageneric clades that were recovered. These numbered clades and subclades are marked in Fig. 2.

Subgenus *Urticocystis* (Clade A): clades A1-A5

Clade A1 (0.57 BPP, / MLB), cheilocystidia mainly of the *brevipes*-type or rarely absent. Stipe striate longitudinally or not.

Subclade A1.1 (1.0 BPP, 100% MLB) (= *M. grammopodia* complex = subsect. *Grammopodiae*). This well supported subclade comprises *M. grammopodia* and *M. grammopodia* f. *macrocarpa* Boekhout, all with urticocystidia, and *M. privernensis* and *M.* sp. 13 without cystidia. All taxa have a distinctly longitudinally striate stipe with a pruinose apex. The eight sequences display a 96.8 P%IV. *Melanoleuca grammopodia* f. *macrocarpa* differs from the type

only by a stipe that is much shorter than pileus diameter (P%IV = 99.4) and so should be considered only a growth form of *M. grammopodia*. *Melanoleuca privernensis* is diagnosed by non-amyloid spores, unique in *Melanoleuca* (Vizzini et al. 2010). The molecular analysis suggests it might represent an aberrant neotenic form of *M. grammopodia* lacking cystidia with non-amyloid spores.

Subclade A1.2 (0.62 BPP, / MLB) includes *M. brevipes* (Bull.) Pat., *M. rasilis* (Fr.) Singer, and two unidentified species (*M.* sp. 14, *M.* sp. 15). *Melanoleuca rasilis* is related to *M. brevipes* based on similar cystidia, grey pileus and stipe colours, and a non-longitudinally striate stipe but differs by smaller basidiomes, a stipe length equal to the pileus diameter, and broadly ellipsoid coarsely ornamented spores (Boekhout 1988, 1999). We also observed that *M. rasilis* has a uniformly white context while *M. brevipes* has a white pileus context and brown stipe context. Molecularly, the two species are clearly different (P%IV = 94.5; Fig. 2).

Subclade A1.3 (1.0 BPP, 96% MLB) comprises *M. sublanipes*, *M.* sp. 16, and *M.* sp. 17. *Melanoleuca sublanipes* (formally described below) is characterized by a tomentose-woolly pileus margin and stipe base. The two *M.* sp. could be conspecific with *M. sublanipes*, but their collections lack of macromorphological data and descriptions. We here propose the following new diagnosis:

Melanoleuca sublanipes Fontenla, Para & Vizzini, **sp. nov.**

MycoBank MB 563140

Pileus -10 cm latus, velutinus, obscure griseo-brunneus, ochraceo-brunneus, griseo-brunneus, a margine pallescens. Lamellae albae, albidae vel pallide griseo-brunneae. Stipes 1.5–7 × 0.3–1.1 cm, lanatus deinde solum inferne lanato-flocculosus, in senectute omnino pruinoso-flocculosus, argenteus, a basi griseo-brunneus, cum ripercussis cyaneis. Caro in pileo albida, in stipite brunnea; odor herbaceus; sapor mitis. Sporae 6.2–9.6 × 3.8–6.6 µm; in medio 7.56 × 5.54 µm; Q = 1.04–1.70; Qm = 1.36, ellipsoideae, conspicuis verrucis singulis, globulosis et amyloideis exornatae. Basidia tetraspora. Cheilocystidia numerosa, 31–55 × 5–8 µm, pili urticae revocantibus, raro fusoidea. Pilei cutis ad marginem ex hyphis erectis, 7–8 µm latis ad instar trichodermatis efformata; hyphae terminales cylindricae, usque ad 100 µm longae. Stipitis cutis pilis clavatis aggregatis et caulocystidiis multiformibus ornata. Habitat in herbidis locis.

Type: Italy, Veneto, Padova, Montegrotto Terme, 07.XI.2008, leg. G. Zecchin (**holotype** ANC M0222).

Etymology: the specific epithet refers to the tomentose-woolly surface of the stipe base.

Clade A2 (1.0 BPP, 96% MLB), cheilocystidia mainly of the *exscissa*-type: subclades A2.1, A2.2 and A2.3.

Subclade A2.1 (0.54 BPP, / MLB) (*M. exscissa* complex) comprises *M. exscissa*, *M. diverticulata*, *M. iris*, and *M. substrictipes* var. *sarcophylla*. The nine sequences display a 98.5 P%IV. This clade reflects an overemphasis on basidiome colour

and smell, with some anatomical traits having led to the establishment of some superfluous species. *Melanoleuca iris* differs from *M. exscissa* only in the strong and sweet smell recalling *Lepista irina* (Fr.) H.E. Bigelow (Kühner 1956, Klán 1983, Bon 1991, Boekhout 1988, 1999; Krieglsteiner 2001, Fontenla et al. 2003). Boekhout (1988, 1999) considered it a variety of *M. exscissa* and Krieglsteiner (2001) cited it as only an accidental *M. exscissa* phenotype; our molecular data (P%IV between *M. iris* and *M. exscissa* sequences = 98.7) clearly support Krieglsteiner. With an intraspecific variability lower than 3% (Nilsson et al. 2008), *M. iris* should be considered a form of *M. exscissa*. *Melanoleuca diverticulata* differs from *M. exscissa* mainly by the presence of elements with small outgrowths in the pileipellis (Moreno & Bon 1980). The P%IV between *M. diverticulata* and *M. exscissa* ANC M0207 and ANC M0208 equals 97.9. Our data show that *M. substrictipes* var. *sarcophylla,* which Kühner (1978) distinguished from the typical variety based on its pinkish lamellae (Kühner 1978, Bon 1991), is more closely related to *M. exscissa* than to *M. substrictipes* Kühner (Fig. 2) and should be considered a form of *M. exscissa* with coloured lamellae (P%IV = 98.5).

Therefore, we propose the following new combinations:

Melanoleuca exscissa f. ***iris*** (Kühner) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563141

≡ *Melanoleuca iris* Kühner, Bull. Soc. Linn. Lyon 25(7): 181 (1956).

The holotype of *M. iris* apparently missing from G (herbarium where Kühner's collections of *Melanoleuca* are kept; Fontenla & Para, unpublished data).

Melanoleuca exscissa f. ***sarcophylla*** (Kühner) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563142

≡ *Melanoleuca substrictipes* var. *sarcophylla* Kühner, Bull. Soc. Linn. Lyon 47(1): 52 (1978).

Holotype (G - K 65-24), with morphological features coincident with those of our sequenced collection (ANC M0209; Fontenla & Para, unpublished data).

Melanoleuca exscissa f. ***diverticulata*** (G. Moreno & Bon) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563143

≡ *Melanoleuca diverticulata* G. Moreno & Bon, Docum. Mycol. 11(41): 35 (1980).

Holotype (MA - 3662), with morphological features coincident with those of our sequenced collection (ANC M0206; Fontenla & Para 2007).

Subclade A2.2 (0.95 BPP, 77% MLB) The two *M. substrictipes* collections are very similar (P%IV = 99.1). The first (ANC M0214) has only urticoid *exscissa*-type cheilocystidia, whereas the second (MCVE 13934) possesses

both *brevipes*- and *exscissa*-type cheilocystidia. Following Bon (1991) the two molecularly clearly conspecific collections would be referred to two different subsections: the first in the subsect. *Exscissae* (as *M. pseudoevenosa* Bon ex Bon & G. Moreno) and the second in subsect. *Grammopodiae*. The holotype (G-K 66-13) is characterized by both types of urticiform cheilocystidia (Fontenla & Para 2011).

Subclade A2.3 (1.0 BPP, 100 % MLB) The two *M. verrucipes* (Fr.) Singer sequences (GenBank DQ490642 and MCVE 09962) are almost identical (P%IV = 99.8). The black dotted stipe readily diagnoses the species (Bon 1991, Boekhout 1999, Gasparini 2001, Fontenla et al. 2003).

Clade A3 (0.53 BPP, / MLB), cheilocystidia absent or urticocystidia mainly of the *brevipes*-type. Both pileus and stipe usually (except for *M. robertiana*) dark coloured and lamellae white to whitish. This clade encompasses the two acystidiate species, *M. robertiana* Bon and *M. stridula* (Fr.) Singer, and subclades A3.1 and A3.2.

Subclade A3.1 (0.66 BPP, / MLB) comprises four acystidiate taxa, *M. striimarginata* Métrod ex Bon, *M. "paratristis," M. graminicola* (Velen.) Kühner & Maire, and *M. angelesiana* A.H. Sm. The first three taxa (subg. *Acystis* sect. *Acystis* in Bon 1991) are morphologically and molecularly (P%IV = 98.2) closely related: they share a collybioid habit, white lamellae, and a whitish stipe, differing mainly in pileus colour and could probably be reduced to only one species, *M. graminicola* (which would have nomenclatural priority). *Melanoleuca angelesiana* (the 2 sequences with a P%IV = 99.9) is an independent species (P%IV = 94.3), distinguished by a tricholomatoid habit, grey lamellae, and greenish-brown stipe. The two Italian collections (ANC M0203, ANC M0204) show features consistent with the protologue (Smith 1944) and holotype (MICH 14633) of this North American species (Fontenla & Para, unpub. data).

Subclade A3.2 (0.99 BPP, 89 % MLB) encompasses *M. decembris* Métrod ex Bon and *M. pseudopaedida* Bon. Bon (1991) classified these species as acystidiate (subg. *Acystis*, sect. *Decembres*), but Fontenla & Para (2008) found numerous urticiform cheilocystidia when they studied the type collections. Both taxa are characterized by a dark (dark brown-grey to blackish) pileus and grey lamellae (Bon 1991). Molecular analysis supports *M. pseudopaedida* as conspecific with *M. decembris* (P%IV = 98.1).

Clade A4 (0.54 BPP, / MLB), cheilocystidia absent or both *brevipes*- and *exscissa*-type urticocystidia, comprising the acystidiate *M. microcephala* (P. Karst.) Singer and A4.1 and A4.2 subclades. Bon (1991) described *M. microcephala* as having urticiform cystidia, but Fontenla and Para (unpub. data) found Karsten's type collection (H 1604) to be acystidiate.

Subclade A4.1 (1.0 BPP, 99 % MLB) contains *M. pseudoluscina*, *M.* sp. 12, and *M.* sp. 18. The seven sequences show a P%IV of 97.1. The P%IV (98.6) between *M.* sp. 18 and *M. pseudoluscina* sequences indicates that *M.* sp. 18 is an acystidiate form of *M. pseudoluscina*. Our analysis support *M. pseudoluscina* as an independent species and not a variety of *M. rasilis* (subclade A1.2) as stated by Boekhout (1988, 1999).

Subclade A4.2 (1.0 BPP, 99 % MLB) encompasses *M. paedida*, *M.* sp. 2, and *M. electropoda*. The two *M. paedida* collections are consistent with the protologue and the observations by Fontenla et al. (2003). *Melanoleuca* sp. 2 is an acystidiate form of *M. paedida*. *Melanoleuca electropoda* was reported by Bon (1991) as a macrocystidiate species (subg. *Melanoleuca*, sect. *Oreineae*); after finding typical urticoid cheilocystidia in the type collection, Fontenla et al. (2009) considered *M. rufipes* Bon a later synonym. As *M. electropoda* differs from *M. paedida* only in an orange red (instead of brown) stipe base and context, we reduce it to a form of *M. paedida* based on molecular data (P%IV = 98.9).

Melanoleuca paedida f. ***electropoda*** (Maire & Malençon) Fontenla, Para & Vizzini, **comb. nov.**, **stat. nov.**

MycoBank MB 563144

≡ *Melanoleuca electropoda* Maire & Malençon, Fl. Champ. sup. Maroc 2: 77 (1975).

Clade A5 (1.0 BPP, 99% MLB) (= sect. *Cognatae*), macrocystidiate taxa with bright coloured pilei.

Melanoleuca cognata (Fr.) Konrad & Maubl. and *M. arcuata* (Bull.) Singer are two independent species (P%IV = 95.4). *Melanoleuca arcuata* differs by having a brown pileus and yellow-ochre lamellae only at maturity. The collection named *M.* sp. 10 is probably referable to a new species (P%IV = 93.1), but its macromorphological data are lacking.

Subgenus *Melanoleuca* (Clade B): clades B1-B5 + *M. friesii* (Fr.) Singer (a species characterized by a dark-brown blackish, slate-grey pileus, whitish lamellae and mainly lageniform cystidia).

Clade B1 (1.0 BPP, 95% MLB), macrocystidia both fusiform and lageniform, pileus mainly grey-brown and lamellae white to grey. It encompasses the subclades B1.1, B1.2, B1.3 + 4 *M.* sp. (*M.* sp. 3, *M.* sp. 4, *M.* sp. 5 and *M.* sp. 11).

Subclade B1.1 (0.79 BPP, 75 % MLB) comprises *M. subpulverulenta* (Pers.) Singer, *M. melaleuca* and *M. nivea* 1. The four collections are closely related (P%IV = 98.6) despite their morphological differences. *Melanoleuca subpulverulenta* is characterized by a pruinose matte grey pileus, pruinose stipe,

and dark context; *M. melaleuca* has a grey-brown non-pruinose pileus, non-pruinose stipe, and whitish context sometimes darkening towards the stipe base. The *M. nivea* 1 collection (ANC M0177) we originally referred to *M. nivea* due to its whitish colours (sect. *Alboflavidae*) and <5 cm pileus represents only an albinic form of the *M. subpulverulenta*/*M. melaleuca* complex; its features nonetheless match those of the *M. nivea* holotype (PC–2434) (Fontenla & Para, unpublished data).

Subclade B1.2 (0.56 BPP, 45 % MLB) comprises *M.* sp. 6, *M. nivea* 3, and *M. robusta*, with the latter distinguished by a grey-brown pileus, grey lamellae, brown context, caespitose growth, and mainly fusiform macrocystidia. *Melanoleuca nivea* 3 is very close to an unidentified collection (*M.* sp. 6) characterized by a grey pileus.

Subclade B1.3 (0.62 BPP, 73 % MLB) consists of two molecularly closely related taxa (P%IV = 98.8), *M. "lanipes"* and *M. heterocystidiosa* (Beller & Bon) Bon that have both lageniform and fusiform macrocystidia. *Melanoleuca lanipes,* which has dark grey-brown lamellae, strongly longitudinally striate dark grey stipe covered with woolly flocci, and blackish stipe base, resembles *M. cognata* but with a tomentose-wooly stipe.

Clade B2 (0.52 BPP, / MLB), mainly lageniform cystidia, longitudinally striate and brown to dark brown stipe.

Clade B2 is formed by *M. albifolia*, *M. atripes*, *M. nivea* 2, *M.* sp. 1 and *M.* sp. 8. All six sequences are closely related (P%IV = 97.6). Morphologically, *M. albifolia* is well characterized by a rather small size (2.5-5 cm diam), dark sepia-brown pileus, white lamellae, grey-brown stipe, and lageniform cystidia (Boekhout 1988, 1999; Bon 1991); *M. atripes* has a hygrophanous blackish brown pileus, dark brown stipe, yellowish beige lamellae, and mainly fusiform cystidia (Boekhout 1988, 1999; Bon 1991). In contrast, our *M. atripes* collection (ANC M0180) shows mainly lageniform cystidia.

The sequence analysis did not support any independent species in the clade. *M. nivea* 2 is probably an albinic form of the *M. albifolia*/*M. atripes* complex while *M.* sp. 1 is the only acystidiate collection nested within subgen. *Melanoleuca* (clade B).

Clade B3 (1.0 BPP, 92% MLB), macrocystidia mainly fusiform, basidiome white to cream-whitish (sect. *Alboflavidae*).

Clade B3 comprises *M. strictipes* (P. Karst.) Jul. Schäff., *M. evenosa* (Sacc.) Konrad, and *M. subalpina* (Britzelm.) Bresinsky & Stangl. The five samples are very closely related (P%IV = 98.4) and have characters consistent with those of the original collections. Bon (1991) circumscribed the species in sect. *Alboflavidae* mainly based on Qm value, odour, pileus size, and stipe ornamentation. We

have now examined morphologically the holotype of *M. strictipes* (H 2432, Fontenla & Para, unpubl.), lectotype of *M. subalpina* (Fontenla & Para 2008), and eight original *Tricholoma cnista* sensu Bresadola (= *M. evenosa*) collections (TR, BPI). This, combined with our thorough study of the original diagnoses and phylogenetic analysis, lead us to conclude that *M. strictipes*, *M. evenosa,* and *M. subalpina* are conspecific, with the name *M. strictipes* having priority.

Clade B4 (0.52 BPP, / MLB), cystidia fusiform or lageniform, pileus small (up to 5 cm broad) and not dark-coloured, lamellae whitish-cream, context white.

Clade B4 comprises *M. oreina* (Fr.) Kühner & Maire, *M. bataillei* Malençon, *M.* sp. 7, and *M.* sp. 9. The first two species, which are phenetically very similar, differ mainly on cystidial shape. Bon (1991) placed *M. oreina* (with fusiform cystidia) into sect. *Oreinae* and *M. bataillei* (with mainly lageniform cystidia) into sect. *Melanoleuca*. Molecularly, they are quite distinct (P%IV = 92.5).

Clade B5 (0.84 BPP, 54% MLB) consists of the three *M. cinereifolia* (Bon) Bon sequences (P%IV = 100).

Melanoleuca cinereifolia is distinguished by a habit reminiscent of *Clitocybe nebularis* (Batsch) P. Kumm., always grey lamellae, short stipe, strictly lageniform (M2) cystidia, and growth in sand dunes.

Acknowledgements

We are grateful to G. Robich (Venice) for having sent us the *Melanoleuca* samples from MCVE DNA bar-coded by Dr. M. Garbelotto (University of California, Berkeley). Dr. Matteo Garbelotto and Dr. Todd Osmundson (University of California, Berkeley) kindly provided us with pre-submission *Melanoleuca* sequences. We also thank Dr. Vladimír Antonín (Brno, Czech Republic) and Dr. Marco Contu (Olbia, Italy) for their pre-submission reviews and Dr. Shaun Pennycook (Auckland, New Zealand) for the nomenclatural review.

Literature cited

Binder M, Hibbett DS, Wang Z, Farnham W. 2006. Evolutionary relationships of *Mycaureola dilseae* (*Agaricales*), a basidiomycete pathogen of a subtidal rhodophyte. Am. J. Bot. 93: 547–556. http://dx.doi.org/10.3732/ajb.93.4.547

Bodensteiner P, Binder M, Moncalvo JM, Agerer R, Hibbett DS. 2004. Phylogenetic relationships of cyphelloid homobasidiomycetes. Mol. Phylogenet. Evol. 33: 501–515. http://dx.doi.org/10.1016/j.ympev.2004.06.007

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(4): 397–431.

Boekhout T. 1999. *Melanoleuca*. 153–165, in: C Bas et al. (eds). Flora Agaricina Neerlandica 4. A.A. Balkema, Rotterdam.

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

Bon M. 1991. Flore mycologique d'Europe, 2 – Les Tricholomes et ressemblants. Doc. Mycol., Mém. hors-Sér. 2: ii, 163 p.

Bresinsky A. 2006. Observations on Mycobiota in Estonia. Folia Cryptogamica Estonica 42: 1–9.

Deschuyteneer D. 2008. Contribution à l'étude de *Melanoleuca nivea*. Revue du Cercle de Mycologie de Bruxelles 8: 57–64.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2010. Geneious v5.3. [Available from http://www.geneious.com].

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. http://dx.doi.org/10.2307/2408678

Fontenla R, Para R. 2007. Osservazioni sul genere *Melanoleuca*. *S*tudio dei tipi I. Rivista di Micologia 50(3): 221–236.

Fontenla R, Para R. 2008. Osservazioni sul genere *Melanoleuca*. Studio dei tipi II. Rivista di Micologia 51(2): 147–162.

Fontenla R, Para R. 2011. Observations on *Melanoleuca*. Type studies – 3. Mycotaxon 115: 215–226. http://dx.doi.org/10.5248/115.215

Fontenla R, Gottardi M, Para R. 2003. Osservazioni sul genere *Melanoleuca*. Fungi non delineati. Pars XXV. Ed. Candusso. Alassio.

Fontenla R, Gottardi M, Para R. 2009. Il genere *Melanoleuca*. 399–405, in: J-C Maire et al. (eds). Compléments à la Flore des champignons supérieurs du Maroc de G. Malençon et R. Bertault. Confédération Européenne de Mycologie Méditerranéenne, Nice.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2(2): 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gasparini G. 2001. *Melanoleuca verrucipes*: una rara specie segnalata anche in Italia. Rivista di Micologia 44(2): 171–174.

Gillman LS, Miller OK. 1977. A study of the boreal, alpine, and arctic species of *Melanoleuca*. Mycologia 69: 927–951. http://dx.doi.org/10.2307/3758777

Horak E. 2005. Röhrlinge und Blätterpilze in Europa. Spektrum, Elsevier, Heidelberg.

Huelsenbeck JP, Larget B, Miller RE, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 5: 673–688.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Justo A, Vizzini A, Minnis AM, Menolli Jr N, Capelari M, Rodriguez O, Malysheva E, Contu M, Ghingnone S, Hibbett DS. 2011. Phylogeny of the *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution. Fungal Biology 115: 1–20. http://dx.doi.org/10.1016/j.funbio.2010.09.012

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30: 3059–3066. http://dx.doi.org/10.1093/nar/gkf436

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth and Bisby's dictionary of the fungi. 10th ed. CABI, Wallingford.

Klán J. 1983. *Melanoleuca iris* in Czechoslovakia (*Agaricales*, *Tricholomataceae*). Česká Mykologie 37(1): 52–55.

Krieglsteiner GJ. 2001. Die Grosspilze Baden-Würtembergs vol. 3. Stuttgart.

Kühner R. 1956. Un *Melanoleuca* parfumé: *M. iris* sp. nov. et l'espèce voisine: *M. excissa* (Fr.) Bull. Soc. Linn. Lyon 25: 176–181.

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

Matheny PB, Curtis JC, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of *Agaricales*: a multi-locus phylogenetic overview. Mycologia 98: 982–995. http://dx.doi.org/10.3852/mycologia.98.6.982

Métrod G. 1942. Sur le genre *Melanoleuca*. Revue Mycol. 7(2–4): 89–96.

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

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49: 278–305. http://dx.doi.org/10.1093/sysbio/49.2.278

Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller OK. 2002. One hundred and seventeen clades of euagarics. Mol. Phylogenet. Evol. 23: 357–400. http://dx.doi.org/10.1016/S1055-7903(02)00027-1

Moreno G, Bon M. 1980. Quelques espèces intéressantes ou nouvelles du genre *Melanoleuca* récoltées en Espagne. Doc. Mycol. 11(41): 35–46.

Moser M. 1983. Die Röhrlinge und Blätterpilze. 5th ed. Kleine Kryptogamenflora, Band II b/2. G. Fischer, Stuttgart, New York.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H. 2008. Intraspecific ITS variability in the Kingdom Fungi as expressed in the International Sequence Databases and its implications for molecular species identification. Evol. Bioinf. 4: 193–201.

Pegler DN, Young TWK. 1973. Basidiospore form in the British *Leucopaxilleae*. Kew Bulletin 28(3): 365–379. http://dx.doi.org/10.2307/4108880

Pfister J. 1984. Études des types de Peck et de Murrill appartenant ou ayant appartenu au genre *Melanoleuca*. Mycotaxon 19: 101–132.

Posada D. 2008. jModeltest: phylogenetic model averaging. Mol. Biol. Evol. 25: 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Rambaut A, Drummond AJ. 2007. Tracer v1.4. [Available from http://beast.bio.ed.ac.uk/Tracer].

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

Singer R. 1943. Das system der *Agaricales* III. Annales Mycologici 41: 1–189.

Singer R. 1986. The *Agaricales* in modern taxonomy, 4th edn. Koeltz Scientific Books, Koenigstein.

Smith AH. 1944. New North American agarics. Mycologia 36(3): 242–262. http://dx.doi.org/10.2307/3754821

Stamatakis A. 2006. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. http://dx.doi.org/10.1093/bioinformatics/btl446

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. (in press). http://dx.doi.org/10.1093/molbev/msr121

Thiers B. 2011. (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/

Vesterholt J. 2008. *Melanoleuca* Pat. 347–352, in: H Knudsen, J. Vesterholt (eds). Funga Nordica – Agaricoid, boletoid and cyphelloid genera. Nordsvamp, Copenhagen.

Vizzini A, Consiglio G, Setti L, Murat C. 2010. The agaricoid genus *Kinia* is a new member of the Pluteoid clade subordinate to *Melanoleuca*. Mycosphere 1(2): 141–145.

Watling R, Turnbull E. 1998. *Cantharellaceae*, *Gomphaceae* and amyloid-spored and xeruloid members of *Tricholomataceae* (excl. *Mycena*). British Fungus Flora, Agarics and Boleti 8. Royal Botanic Gardens Edinburgh.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols. Academic Press, London.