



Melanoleuca juliannae (Basidiomycota, Tricholomataceae), a new species from subgen. *Urticocystis*

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Abstract

A new species, *Melanoleuca juliannae* is described from Hungary. Its most outstanding character is the presence of a violaceous-blue colour in the stipe base. Sequence comparisons with other *Melanoleuca* species revealed highly similar but decolourate members of the species, lacking the remarkable colour of the stipe. These specimens are described as *Melanoleuca juliannae* var. *decolorans* based on three collections from the Czech Republic and Italy. Detailed macro- and microscopic descriptions of the new species are given. The position of *M. juliannae* within subgen. *Urticocystis* was confirmed by DNA sequence data of the ITS region of the nuclear ribosomal RNA gene cluster and translation elongation factor 1-alpha (*tef1*). Although intra-individual heterogeneity in ITS region was revealed in some specimens of the type variety, the *tef1* sequences lack a similar heterogeneity.

Key words: taxonomy, phylogeny, ITS, *tef1*, Czech Republic, Hungary, Italy

Introduction

A very interesting *Melanoleuca* species was collected on several localities in the Great Hungarian Plain (Eupannonicum). Its most distinctive character was the violaceous-blue colour of the stipe base. The first colour photo and description of the blue-stiped *Melanoleuca* was published in 2008 by Rimóczi (2008). DNA was extracted from the specimens and the sequences of the internal transcribed spacer (ITS) of the nuclear ribosomal RNA gene were determined and subjected to search for similar sequences by BLAST (<http://blast.ncbi.nlm.nih.gov>; accessed on 23 January 2014). The search did not yield a conspecific hit as the closest sequenced species proved to be *Melanoleuca grammopodia* (95% identity). Further, recent papers on *Melanoleuca* (Vizzini *et al.* 2011, Sánchez-García *et al.* 2013) did not recognize this species. The literature on European *Melanoleuca* taxa (Boekhout 1999, Bon 1990, Fontenla *et al.* 2003, Kühner 1978, Métrod 1948, Vesterholt 2012, Watling & Turnbull 1998) did not mention bluish violaceous stipe base within the genus. Also current European experts in *Melanoleuca*, R. Fontenla and R. Para, are unaware of a species having such a character (Para & Fontenla pers. comm.). Comparison with an extensive ITS sequence database of *Melanoleuca* collections (≥ 320 specimens) in the BRNM herbarium and specimens of the 9th author from various European regions and biotopes revealed highly similar or identical sequences of specimens lacking the remarkable blue colour of the stipe. The conspecific specimens lacking blue colour were recognized among collections from the Czech Republic and Italy, but were not referable to any named species. Therefore, we decided to describe a new species with two varieties differing in stipe colour.

6F5, 7E45-6) pileus (Carotti *et al.* 2006, Fontenla *et al.* 2003, Antonín – own observations); Boekhout (1999), Fontenla *et al.* (2003) and Antonín (own observations) mentioned the absence of urticoid caulocystidia, while Carotti *et al.* (2006) admit the presence of scattered caulocystidia. *Melanoleuca humilis* has a 50–120 mm broad pileus, more robust stipe (22–90 × 7–25 mm), and it started to grow early in the year in Central Europe at places often strongly influenced by human activity (Watling & Turnbull 1998, Antonín – own observations). Bon (1990) mentioned the presence of vividly umbra brown to vinaceous pink stipe base in *M. electropus*, but stipe colour is described as vividly umbra brown to rusty in the original description (Malençon & Bertault 1975: 77, as *M. electropoda*). Moreover, this species differs by the umbra olivaceous pileus and smaller basidiospores (7.4–7.6 × 4.8–5.4 µm in the original description, 6–7.4 × 4.8–6 µm by Para & Fontenla pers. comm.).

When we retrieved the first ITS sequence data of intra-individual heterogeneity, we hypothesized that the heterozygotic specimens represent recent hybrid individuals but this was not supported by *tefl* sequence data lacking a similar heterogeneity. Usually the *tefl* sequences are more variable than that of ITS sequences and molecular taxonomy based on ITS sequences alone underestimates the number of species predicted by other nuclear loci (Gazis *et al.* 2011). Also our previous studies revealed higher variability of *tefl* than ITS sequences among related species (Antonín *et al.* 2009, Tomšovský *et al.* 2010). The intragenomic ITS variability resulting in sequence heterogeneity might be caused by heterogeneity between repeats of RNA gene copies within the genome (confirm Lindner & Banik 2011).

The results of detailed macro- and micromorphological and molecular studies by the first and the last author of this study and Vizzini *et al.* (2011) discovered a big overlap of both macro- and microscopic characters. Therefore, further phylogenetic analyses supported by detailed macro- and micromorphological observations are necessary for resolving the taxonomy of the genus *Melanoleuca*.

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References

- Antonín, V., Tomšovský, M., Sedlák, P., Májek, T. & Jankovský, L. (2009) Morphological and molecular characterization of the *Armillaria cepistipes* – *A. gallica* complex in the Czech Republic and Slovakia. *Mycological Progress* 8: 259–271.
<http://dx.doi.org/10.1007/s11557-009-0597-1>
- 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, Th.W., Noordeloos, M.E. & Vellinga, E.C. (eds.) *Flora agaricina neerlandica*. Vol. 4. A.A. Balkema, Rotterdam – Brookfield, pp. 153–165.
- Bon, M. (1990) Les Tricholomes et ressemblant. *Documents Mycologiques Mémoire Hors Série* n. 2: 1–163.
- Bulliard, P. (1792) *Herbier de la France*. Vol. 12. Paris. Tabs. 577–600.
- Carotti, V., Consiglio, G., Contu, M., Fontenla, R., Gottardi, M. & Para, R. (2006) Contributo alla conoscenza dei macromyceti dell'Emilia-Romagna. XXII. Genere *Melanoleuca*. *Bollettino dell'Associazione Micologica ed Ecologica Romana* 22: 9–39.
- Fontenla, R., Gottardi, M. & Para, R. (2003) Osservazioni sul genere *Melanoleuca*. *Fungi non Delineati* 25: 1–112.
- Fries, E.M. (1821) *Systema mycologicum*. I. Gryphiswaldiae. 238 pp.
- Kornerup, A. & Wanscher, J.H. (1983) *Methuen handbook of colour*. Ed. 3. Eyre Methuen, London. 252 pp.
- Gazis, R., Rehner, S. & Chaverri, P. (2011) Species delimitation in fungal endophyte diversity studies and its implications in ecological

- and biogeographic inferences. *Molecular Ecology* 20: 3001–3013.
<http://dx.doi.org/10.1111/j.1365-294x.2011.05110.x>
- Huelsenbeck, J.P. & Ronquist, F. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
<http://dx.doi.org/10.1093/molbev/mst010>
- Kühner, R. (1978) Agaricales de la zone alpine. Genre *Melanoleuca* Pat. *Bulletin de la Société Linnéenne de Lyon* 47: 12–52.
- Lindner, D.L. & Banik, M.T. (2011) Intra-genomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus*. *Mycologia* 103: 731–740.
<http://dx.doi.org/10.3852/10-331>
- Malençon, G. & Bertault, R. (1975) Flore des champignons supérieurs du Maroc. Tome 2. *Travaux de l'Institut Scientifique Cherifien et de la Faculté des Sciences de Rabat, Série Botanique et Biologie Végétale* 33: 1–541.
- Métrod, G. (1948) Essai sur le genre *Melanoleuca* Patouillard emend. *Bulletin de la Société Mycologique de France* 64: 141–165.
- Nikolcheva, L.G. & Bärlocher, F. (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycological Progress* 3: 41–49.
<http://dx.doi.org/10.1007/s11557-006-0075-y>
- Patouillard, N. (1897) Catalogue raisonné des plantes cellulaires de la Tunisie. Fungi. Paris. 19–136 pp.
<http://dx.doi.org/10.5962/bhl.title.3899>
- Persoon, C.H. (1801) *Synopsis Methodica Fungorum*. Gottingae. 745 pp.
<http://dx.doi.org/10.5962/bhl.title.5393>
- Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97(1): 84–98.
<http://dx.doi.org/10.3852/mycologia.97.1.84>
- Rimóczi, I. (2008) *Gombaválogató 9*. Szaktudás Kiadó Ház, Budapest. 136 pp.
- Rimóczi, I., Jeppson, M. & Benedek, L. (2011) Characteristic and rare species of Gasteromycetes in Eupannonicum. *Fungi non delineati* 56–57: 1–226.
- Sánchez-García, M., Cifuentes-Blanco, J. & Matheny, P. B. (2013) Revisión taxonómica del género *Melanoleuca* en México y descripción de especies nuevas. *Revista Mexicana de Biodiversidad*: S111–S127.
<http://dx.doi.org/10.7550/rmb.31569>
- Singer, R. (1939) Phylogenie und Taxonomie der Agaricales. *Schweizerische Zeitschrift für Pilzkunde* 17: 52–57.
- Swofford, D.L. (2003) *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, USA.
- Tomšovský, M., Sedlák, P. & Jankovský, L. (2010) Species recognition and phylogenetic relationships of European *Porodaedalea* (Basidiomycota, Hymenochaetales). *Mycological Progress* 9: 225–233.
<http://dx.doi.org/10.1007/s11557-009-0628-y>
- Tomšovský, M. (2012) Delimitation of an almost forgotten species *Spongipellis litschaueri* (Polyporales, Basidiomycota) and its taxonomic position within the genus. *Mycological Progress* 11(2): 415–424.
<http://dx.doi.org/10.1007/s11557-011-0756-z>
- Thiers, B. (2013, 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: 26 December 2013).
- 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.
<http://dx.doi.org/10.5248/118.361>
- Vizzini, A., Ercole, E., Fontenla, R. & Para, R. (2012) Osservazioni sul genere *Melanoleuca*. *M. humilis* e *M. sublanipes*. *Micologia e Vegetazione Mediterranea* 27(2): 93–108.
- 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 Garden, Edinburgh, 189 pp.