



# The genus *Dermoloma* is more diverse than expected and forms a monophyletic lineage in the Tricholomataceae

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## Abstract

We present the first phylogenetic evaluation of the genus *Dermoloma*, which is resolved as monophyletic and closely related to *Pseudotracheloma*, a poorly known *Dermoloma*-like lineage within the family Tricholomataceae. The position of *Dermoloma* is confirmed by the placement of the type species, *Dermoloma cuneifolium*, represented by multiple samples including the neotype. Based on our phylogenetic analyses, we recognised 25 European operational taxonomic units (OTUs) but could only assign species names to ten of them based on ex-type sequences. Furthermore, only five additional published *Dermoloma* names of uncertain status are available for the remaining 16 potential European species, thus demonstrating an unexpected amount of taxonomic diversity. Samples from Europe and North America seem to be endemic on a continental scale. North American samples formed six unique OTUs, but only one could be reliably named, *Dermoloma hymenocephalum*. *Dermoloma* is morphologically defined by basidiomata with brown, grey and white colours with a farinaceous odour and a pluristratous hymeniderm type of pileipellis. Our phylogenetic analyses support the subdivision of the genus into two subgenera and four sections, and species with inamyloid basidiospores are placed in subg. *Dermoloma* and those with amyloid basidiospores in subg. *Amylospora*. Both subgenera are further divided into two sections. The analysis of spore morphology shows that sect. *Conica* of subg. *Dermoloma* and sect. *Nigrescentia* of subg. *Amylospora* have a very distinctive spore shape. Sect. *Atrobrunnea* of subg. *Amylospora* showed relatively high variability of spores among species, but spores of sect. *Dermoloma* were similar and not useful for species discrimination.

**Keywords** Agaricales · Infrageneric classification · Systematics · Cryptic diversity · CHEGD fungi

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## Introduction

The genus *Dermoloma* (J. Lange) Singer ex Herink is a poorly known group of agarics defined by small- to medium-sized basidiomata of collybioid to tricholomatoid habit with dull grey, brownish or white colourations; sinuate to adnate-decurrent lamellae; farinaceous smell (or taste); smooth, hyaline, amyloid or inamyloid basidiospores; and a pileipellis of a pluristratuous (multi-layered) hymeniderm type (Arnolds 1992; Singer 1975, 1986). Thirty-nine unique names at species and lower rank have been published or combined in the genus (<http://www.mycobank.org>, <http://www.indexfungorum.org>). Among them, 25 were published from Europe, six from the Americas and eight from other continents. Among European names, four are invalid or illegitimate and three are infraspecific taxa. This means that 18 valid species names are published from Europe (Table 1).

The number of accepted species has varied over the years in Europe. Bon (1986, 1998) accepted eight species, but only three were adopted by Arnolds (1992, 1993), who later added one more European species (Arnolds 2002). It is not clear if Contu et al. (2008) accepted all previously described European species as separate taxa, but they considered that the diversity of European *Dermoloma* was higher, and described four new species, one of which was not validly published. Only three species are included in a recent key of Nordic mycobiota (Vesterholt 2008, 2012). European species are mostly found in grassland ecosystems and are part of a group of fungi with special conservation interest that has been referred to as CHEGD (acronym of taxon names with the last letter referring to *Dermoloma*) (Griffith et al. 2013). The diversity of the genus in North America has not been explored, and only a single species, *Dermoloma hymenocephalum* (A.H. Smith) Singer, has been described from the USA (Singer 1962).

*Dermoloma* is classified in the family Tricholomataceae R. Heim ex Pouzar ([www.mycobank.org](http://www.mycobank.org)) based on morphological characters (Singer 1975, 1986). Bon (1979) proposed an alternative morphology-based placement based on the pileipellis structure in the family Dermolomataceae (Bon) Bon, but this was not generally accepted. The first phylogenetic study that included a member of the genus placed *Dermoloma inconspicuum* Dennis close to the genus *Lepiota* (Pers.) Gray in the family Agaricaceae Chevall (Kropp 2008). Sánchez-García and Matheny (2016) recently placed five *Dermoloma* samples in the family Tricholomataceae, within a clade sister to the genus *Tricholoma* (Fr.) Staude. In their phylogenetic tree, the monophyletic genus *Pseudotracheloma* (Singer) Sánchez-García and Matheny is nested within *Dermoloma*, making the latter paraphyletic. *Dermoloma* species are traditionally grouped into two sections: *D.* sect. *Dermoloma* with inamyloid basidiospores and *D.* sect. *Atrobrunnea* with amyloid basidiospores (Contu et al. 2008).

Different opinions about classification at family rank suggest that the morphological concept of the genus *Dermoloma* might correspond to several unrelated phylogenetic lineages. Variable species concepts in the European literature focused on spore characters, in combination with additional field characters, suggest that species taxonomy needs extensive revision. Here, we produce the first phylogenetic study of the genus. Our aims are to (i) verify if morphological circumscription of the genus corresponds to a monophyletic group; (ii) define the phylogenetic placement of the genus, including morphological and phylogenetic limits; (iii) estimate if species diversity in Europe and North America correspond to current taxonomic opinions; (iv) assign species names to clades fixed by sequencing type collections; and (v) specify phylogenetic signal and taxonomic relevance of spore characters.

## Material and methods

### Taxon sampling

The study combined type material and other samples collected or gathered by the authors. We were able to borrow 20 of 23 existing *Dermoloma* types from Europe and North America (Table 2). Recent material consisted of additional 87 specimens collected from Estonia (1 collection), France (14), Italy (2), Romania (8), Slovakia (26), Switzerland (1), UK (33) and the USA (2), and 21 borrowed herbarium specimens from Italy (9), Sweden (4) and the USA (8). *Dermoloma* samples collected by the authors of this study were identified based on agaricoid basidiomata of grey, brown and white colours, hymeniderm pileipellis and usually conspicuous farinaceous odour (Arnolds 1992).

### DNA extractions and sequencing

A small piece of dried material (10–30 mg) was removed and ground in a 1.5-ml tube with sterilized sand, liquid nitrogen and a micropestle. Genomic DNA was extracted using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) and the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek) following the manufacturer's instructions. Genomic DNA was serially diluted into two 1:10 dilutions with sterile water. The following loci were targeted: (i) the internal transcribed spacer regions of nuclear ribosomal DNA (nrITS), (ii) nuclear ribosomal large subunit (nrLSU), (iii) the first largest subunit of RNA polymerase II (*rpb1*) and (iv) the most variable region between domains 6 and 7 of the nuclear gene encoding the second largest subunit of RNA polymerase II (*rpb2*). Amplification of DNA was performed using a PCR mix consisting of approximately 2 ng/μl of template DNA, forward and reverse primers (10 pmol/μl), 5× HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu,

**Table 1** List of all species and lower rank taxa published or combined in the genus *Dermoloma* sorted by continent with information of the type country of origin

Africa: 1 name	1. <i>Dermoloma griseocarneum</i> Pegler, <i>Kew Bulletin Additional Series</i> 6: 213 (1977)
Holotypus: Uganda	
America: 6 names	2. <i>Dermoloma atrobrunneum</i> (Dennis) Singer ex Bon, <i>Documents Mycologiques</i> 17(65): 51 (1986)
	≡ <i>Tricholoma atrobrunneum</i> Dennis, <i>Transactions of the British Mycological Society</i> 34: 476 (1951)
	– <i>Dermoloma atrobrunneum</i> (Dennis) Singer, <i>Sydowia</i> 9(1–6): 375 (1955)
Holotypus: Trinidad and Tobago (Trinidad Isl.)	
	3. <i>Dermoloma aposcenum</i> Singer, <i>Fieldiana Botany</i> 21: 88 (1989)
Holotypus: Mexico	
	4. <i>Dermoloma hymenocephalum</i> (A.H. Sm.) Singer, <i>Sydowia</i> 15(1–6): 142 (1962)
	≡ <i>Collybia hymenocephala</i> A.H. Sm., <i>Papers of the Michigan Academy of Sciences</i> 26: 61 (1941)
	≡ <i>Hydropus hymenocephalus</i> (A.H. Sm.) Redhead, <i>Sydowia</i> 37: 266 (1984)
Holotypus: USA	
	5. <i>Dermoloma inconspicuum</i> Dennis, <i>Kew Bulletin</i> 15(1): 78 (1961)
Holotypus: Venezuela	
	6. <i>Dermoloma pataguae</i> Singer, <i>Fieldiana Botany</i> 21: 89 (1989)
Holotypus: Chile	
	7. <i>Dermoloma yungense</i> Singer, <i>Beihefte zur Sydowia</i> 7: 61 (1973)
Holotypus: Bolivia	
Asia: 4 names	8. <i>Dermoloma cystidiatum</i> Manim. & Arnolds, <i>Persoonia</i> 17(1): 149 (1998)
Holotypus: India	
	9. <i>Dermoloma indicum</i> K.N.A. Raj & Manim., <i>Phytotaxa</i> 177 (4): 239 (2014)
Holotypus: India	
	10. <i>Dermoloma keralense</i> K.N.A. Raj & Manim., <i>Phytotaxa</i> 177 (4): 241 (2014)
Holotypus: India	
	11. <i>Dermoloma scotodes</i> (Berk. & Broome) Pegler, <i>Kew Bulletin Additional Series</i> 12: 182 (1986)
	≡ <i>Agaricus scotodes</i> Berk. & Broome, <i>Botanical Journal of the Linnean Society</i> 11(56): 522 (1871)
Holotypus: Sri Lanka	
Australia and Oceania: 3 names	12. <i>Porpoloma amyloideum</i> (G. Stev.) E. Horak, <i>New Zealand Journal of Botany</i> 9(3): 407 (1971)
	≡ <i>Dermoloma amyloideum</i> (G. Stev.) G. Stev., <i>Field Guide to Fungi</i> : 73 (1982)
	≡ <i>Tricholoma amyloideum</i> G. Stev., <i>Kew Bulletin</i> 19(1): 15 (1964)
Holotypus: New Zealand	
	13. <i>Dermoloma hemisphaericum</i> (G. Stev.) E. Horak, <i>New Zealand Journal of Botany</i> 9(3): 429 (1971)
	≡ <i>Tricholoma hemisphaericum</i> G. Stev., <i>Kew Bull.</i> 19(1): 14 (1964)
Holotypus: New Zealand	
	14. <i>Dermoloma murinum</i> (G.M. Taylor & G. Stev.) E. Horak, <i>New Zealand Journal of Botany</i> 9(3): 438 (1971)
	≡ <i>Tricholoma murinum</i> G.M. Taylor & G. Stev., <i>Kew Bulletin</i> 19(1): 17 (1964)
Holotypus: New Zealand	
Europe: 21 valid names and 4 invalid names	15. <i>Dermoloma alexandri</i> Cons., <i>Micologia e Vegetazione Mediterranea</i> 22(2): 84 (2008)
Holotypus: Italy	
	16. <i>Dermoloma atrocinerum</i> (Pers.) P.D. Orton, <i>Transactions of the British Mycological Society</i> 43(2): 175 (1960)
	≡ <i>Agaricus atrocinerus</i> Pers., <i>Synopsis methodica fungorum</i> : 348 (1801)
	– <i>Dermoloma atrocinerum</i> (Pers.) Herink, <i>Sb. severočeského Musea</i> , <i>Historia Naturalis</i> 1: 62 (1958)
Neotypus designated by Contu, Consiglio & Setti (2008): Italy	
	17. <i>Dermoloma bellerianum</i> Bon, <i>Documents Mycologiques</i> 28(nos. 109–110): 6 (1998)
Holotypus: France	
	18. [ <i>Dermoloma cheilocystidiatum</i> Contu, <i>Bollettino dell'Associazione Micologica ed Ecologica Romana</i> 15(no. 48): 4 (2000), nom. inval. published as nomen provisorium]
Authentic material: Italy	
	19. <i>Dermoloma coryleti</i> Singer & Cléménçon, <i>Schweizerische Zeitschrift für Pilzkunde</i> 49(9): 120 (1971)

**Table 1** (continued)

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Holotypus: Czech Republic  
 20. ***Dermoloma cuneifolium*** (Fr.: Fr.) Bon, *Documents Mycologiques* 17(65): 51. 1986  
 – *Agaricus cuneifolius* Fr., *Observationes mycologicae* 2: 99 (1818)  
 Neotypus designated by Arnolds (1992): Sweden  
 21. ***Dermoloma cuneifolium*** var. *punctipes* Arnolds, *Persoonia* 14(4): 529 (1992)

Holotypus: The Netherlands  
 22. ***Dermoloma emiliae-dlouhyi*** Svrček, *Česká Mykologie* 20(3): 147 (1966)

Holotypus: Czech Republic  
 23. ***Dermoloma fuscobrunneum*** P.D. Orton, *Notes from the Royal Botanical Garden Edinburgh* 38(2): 326 (1980)

Holotypus: UK  
 24. [*Dermoloma glauconitens* (Fr.) Bon, *Documents Mycologiques* 17(65): 51. (1986) nom. illegit. (superfluum)]  
 ≡ *Agaricus glauconitens* Fr., *Syst. Mycol.* 3: 22. 1832.  
 – *Agaricus nitens* Batsch, *Elenchus fungorum. Continuatio secunda*: 21. 1789; Fr., *Syst. Mycol.* 1: 116. 1821  
 25. ***Dermoloma hybridum*** (Kühner) Bon, *Bulletin Annual de la Fédération Centre-Est d'Histoire Naturelle et de Mycologie* 1: 14 (1979)  
 ≡ *Tricholoma hybridum* Kühner, *Ann. Sci. Franche-Comté* 2: 31 (1947)

Holotypus: France  
 26. ***Dermoloma hygrophorus*** Joss., *Bull. Soc. Linn. Lyon* 39(1): 6 (1970)  
 ≡ *Tricholoma hygrophorus* Joss., *Bulletin trimestriel de la Société Mycologique de France* 74(4): 482 (1958)

Holotypus: France  
 27. ***Dermoloma intermedium*** Bon, *Documents Mycologiques* 9(35): 42 (1979)

Holotypus: France  
 28. ***Dermoloma intermedium*** var. *coniferarum* Bon, *Documents Mycologiques* 17(65): 51 (1986)

Holotypus: France  
 29. ***Dermoloma josserandii*** Dennis & P.D. Orton, *Transactions of the British Mycological Society* 43(2): 226 (1960)

Holotypus: UK  
 30. *Dermoloma longibasidiatum* Cons., Contu & Setti, *Micologia e Vegetazione Mediterranea* 22(2): 110 (2008)

Holotypus: Italy  
 31. ***Dermoloma magicum*** Arnolds, *Persoonia* 17(4): 665 (2002)

Holotypus: The Netherlands  
 32. ***Dermoloma murinellum*** E. Horak, *Sydowia* 39: 110 (1987)

Holotypus: Switzerland  
 33. [*Dermoloma nitens* Fr. ex Raithehl., *Metrodiana* 8(2–3): 52 (1979), nom. inval.]  
 34. ***Dermoloma phaeopodium*** P.D. Orton, *Notes from the Royal Botanical Garden Edinburgh* 28(2): 327 (1980)  
 ≡ *Dermoloma josserandii* var. *phaeopodium* (P.D. Orton) Arnolds, *Persoonia* 15(2): 195 (1993)

Holotypus: UK  
 35. ***Dermoloma pragense*** Kubička, *Česká Mykologie* 29(1): 31 (1975)  
 ≡ *Dermoloma pseudocuneifolium* var. *pragense* (Kubička) Bon, *Documents Mycologiques* 17(65): 52 (1986)

Holotypus: Czech Republic  
 36. ***Dermoloma pragense*** f. *obscurum* Cons. & Contu, *Micologia e Vegetazione Mediterranea* 22(2): 99 (2008)

Holotypus: Italy  
 37. ***Dermoloma pseudocuneifolium*** Herink ex Bon, *Documents Mycologiques* 17(65): 52 (1986)

Holotypus: France  
 38. ***Dermoloma pusillum*** Contu, *Micologia e Vegetazione Mediterranea* 22(2): 105 (2008)

Holotypus: Italy  
 39. [*Dermoloma simulatum* Cons., Contu & Setti, in Contu, Consiglio & Setti, *Micologia e Vegetazione Mediterranea* 22(2): 110. 2008, nom. inval. described as 'ad int.']  
 Authentic material: Italy

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Names in bold-italics are validly published, and names in brackets are invalid

Estonia) and molecular grade water added up to 20 µl, or using a mixture of 5× buffer, GoTaq and dNTPs supplied by Invitrogen Corp. (Carlsbad, CA, USA). All PCRs started with an initial denaturation step at 95 °C for 15 min.

The nrITS was amplified with the primers ITS1F-ITS4 (Gardes and Bruns 1993; White et al. 1990). Reaction conditions consisted of denaturation at 95 °C for 35 s, annealing at 55 °C for 55 s and elongation at 72 °C for 45 s, then followed

**Table 2** List of European and North American type and authentic herbarium material and sequencing results

Species	Collection details	Herbarium	Type status	Sequencing outputs
<i>D. alexandri</i> Cons.	Italy; Mesole (FE), Boscone della Mesola, G. Consiglio, M. Panchetti, R. Bolletta & C. Orlandini, 12 Sep 2004	Herbarium of G. Consiglio (GC04317)	HT	MW193776
<i>D. atrocinerum</i> (Pers.) P.D. Orton	Italy; Villetta Barrea (AQ), Abruzzo, M. Contu et al., 19 Sep 2003	AQUI (Contu 19.IX.2003)	NT	MW193803
<i>D. bellerianum</i> Bon	France; Beller, 30 Sep 1972	LIP (Bon 912)	HT	Not located
<i>D. coryleti</i> Singer & Cléménçon	Czechia; Kroměříš, R. Singer, 30 Sep 1970	F (R. Singer C-5230)	HT	Not accessed
<i>D. cuneifolium</i> (Fr.: Fr.) Bon	Sweden; Småland, Femsjö, 'Avaberget', S. Lundell & G. Haglund, 19 Sep 1948	UPS F-631065	NT	MW193843
<i>D. cuneifolium</i> var. <i>punctipes</i> Arnolds	Netherlands; prov. Limburg, Wijlre, 'Wrakelberg', E. Arnolds, 22 Oct 1984	L 0821553	HT	Failed
<i>D. emiliae-dlouhyi</i> Svrček	Czechia; Brdské hřebeny Mts., Vižina, M. Svrček, 20 Sep 1965	PR 610931	HT	Failed
<i>D. fuscobrunneum</i> P.D. Orton	UK; Somerset, Bickham Wood, Crawley, P.D. Orton, 24 Oct 1975	E 16876	HT	Failed
<i>D. hybridum</i> (Kühner) Bon	France; bois d'Avoudrey (près Besançon), R. Kühner, 16 Oct 1946	G 126676	HT	Failed
<i>D. hygrophorus</i> Joss.	France; Ain, Quincieu, Lacombe & Josserand, 14 Aug 1956	GG00127362	HT	MW193845, partial ITS sequence
<i>D. hymenocephalum</i> (A.H. Sm.) Singer	USA; Michigan, Dexter, Silver lake, A.H. Smith, 23 Sep 1938	MICH 10228	HT	MW193846, partial ITS sequence
<i>D. intermedium</i> Bon	France; Somme, Warlus, towards Airaines, M. Bon, Oct 1967	LIP (Bon 71081)	HT	Failed
<i>D. intermedium</i> var. <i>coniferarum</i> Bon	France; Argol, Momand, 28 Oct 1982	LIP (Bon 8116)	HT	sequencing successful, not <i>Dermoloma</i>
<i>D. josserandii</i> Dennis & P.D. Orton	UK; South Somerset, Spaxton, Hawkridge, E. Murrigae, 15 Sep 1958	K(M) 37580	HT	Failed
<i>D. longibasidiatum</i> Cons., Contu & Setti	Italy; Pergine (TN), Susà, G. Consiglio, G. Marasca et B. Oss-Emer, 30 Nov 1993	Herbarium of G. Consiglio (GC93318)	HT	Failed
<i>D. magicum</i> Arnolds	Netherlands; Limburg, Epen, Cotessen, E. Arnolds, 21 Oct 1995	L (Arnolds 6701)	HT	Not located
<i>D. murinellum</i> E. Horak	Switzerland; Graubünden, N des Albulpasses (Terrassas), E. Horak, 30 Aug 1982	ZT Myc 42786 (Horak ZT1573)	HT	Failed
<i>D. phaepodium</i> P.D. Orton	UK; Devon, Membury, P.D. Orton, 28 Oct 1977	E 16877	HT	MW193852
<i>D. pragense</i> Kubička	Czechia; Praha, „Kinského sady“, E. Wichanský 22 Jun 1965	PR 611173	HT	Failed
<i>D. pragense</i> f. <i>obscurum</i> Cons. & Contu	Italy; Monte Grino, near Piobbico (PU), G. Consiglio & M. Maletti, 22 Oct 2006	Herbarium of G. Consiglio (GC06186)	HT	MW193853
<i>D. pseudocuneifolium</i> Herink ex Bon	France; near Saint-Valery-sur-Somme, Oct 1968	LIP (Bon 81006)	HT	Failed
<i>D. pusillum</i> Contu	Italy; Sardinia, Olbia (OT), Pittolungu, M. Contu, 30 Dec 2006	AQUI (Contu 30.XII.2006)	HT	MW193861
<i>D. simulatum</i> Cons., Contu & Setti, nom. inval.	Italy; Trento-Alto Adige, Susà (TN), G. Consiglio, G. Marasca & B. Oss-Emer, 30 Oct 1993	Herbarium of G. Consiglio (GC02284)	AM	Failed

Codes starting with MW are GenBank numbers

HT holotype, NT neotype, AM authentic material

by 13 cycles of denaturation at 95 °C for 35 s, annealing at 55 °C for 55 s and elongation at 72 °C for 2 min and the last 9 cycles with the same conditions for denaturation and annealing, with a longer elongation of 3 min. A final extension was carried out at 72 °C for 10 min. The nrLSU was

amplified using the primer pairs LR0R/LR16, LR0R/LR5 or LR0R/LR7 (Vilgalys and Hester 1990, [https://sites.duke.edu/vilgalyslab/rdna\\_primers\\_for\\_fungi/](https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/)). The PCR conditions were as follows: 1 min at 95 °C, 1 min at 50 °C, 1 min at 72 °C, repeated 35 times, and a final extension step for

10 min at 72 °C. The primers bRPB2-6F and bRPB2-7.1R (Matheny 2005) were used to amplify the *rpb2* region. PCR conditions were as follows: 1 min at 95 °C, 1 min at 58 °C, an increase of 1 °C per 5 s to 72 °C and 1 min at 72 °C, repeated 35 times and finalized for 10 min at 72 °C. The *RPB1* region was amplified using the primers gRPB1-A for and fRPB1-C rev (Matheny et al. 2002) under the following PCR conditions: 1 min at 95 °C, 1 min and 30 s at 55 °C, an increase of 1 °C every 5 s to 72 °C and 2 min at 72 °C, repeated 35 times and finalized for 10 min at 72 °C.

The targeted fragments were purified using a PCR Purification Kit (Qiagen, Hilden, Germany). Sequence reactions were performed with BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA, USA) and purified using Sephadex G-50 columns (General Electric Healthcare, Piscataway, NJ, USA). Sequencing was performed at the University of Tennessee Genomics Core and at the SEQme sequencing company (Dobříš, Czech Republic).

### Sequence editing and phylogenetic analyses

Sequence files were edited in Geneious (version R10) (Kearse et al. 2012). Intra-individual polymorphic sites having more than one signal were marked with NC-IUPAC ambiguity codes. For phylogenetic placement of the genus *Dermoloma* within the family Tricholomataceae, we used *Dermoloma* samples represented by sequences of at least two DNA loci supplemented with sequences of the genera *Albomagister* Sánchez-García, Birkebak & Matheny; *Corneriella* Sánchez-García; *Dennisiomyces* Singer; *Leucopaxillus* Boursier; *Porpoloma* Singer; *Pseudobaeospora* Singer; *Pseudotracheloma*; and *Tricholoma* and an undetermined genus published by Sánchez-García et al. (2014) and Sánchez-García and Matheny (2016). *Pseudoomphalina kalchbrenneri* LAS06/037 was used as the outgroup. We performed an independent analysis of the genus *Dermoloma* adding also internal transcribed spacer (ITS) sequences retrieved from material of low-quality DNA including the types. The clade that was inferred as a sister to *Dermoloma* in the Tricholomataceae analysis was used as the outgroup in the *Dermoloma* tree. This second analysis allowed us to identify species clades based on the placement of the type specimens and to estimate more accurately the diversity of the genus. The datasets were aligned in MAFFT 7 using the E-INS-i strategy (Katoh and Standley 2013) and manually improved in Geneious R10 (Kearse et al. 2012). Divergent and ambiguously aligned positions of ITS and large subunit (LSU) were removed with Gblocks (Castresana 2000) using the least stringent parameters. Intronic positions of *rpb1* and *rpb2* were manually removed. Final alignment files are uploaded to TreeBase (#27219 for Tricholomataceae analysis and #27216 for *Dermoloma* analysis).

The final multi-locus datasets were analysed using the CIPRES Science Gateway (Miller et al. 2010) with two different methods: Bayesian inference (BI) and maximum likelihood (ML). For the ML analyses, the concatenated alignments were uploaded as FASTA files and analysed using RAXML-HPC2 on XSEDE (8.2.12) (Stamatakis 2014) as a partitioned dataset under the GTR + GAMMA model with 1000 bootstrap iterations. For the BI analysis, the dataset was divided into ten partitions: ITS1, 5.8S, ITS2, LSU and the 1st, 2nd and 3rd codon positions of *rpb1* and *rpb2*. The best substitution model for each partition was computed jointly in PartitionFinder 1.1.1 (Lanfear et al. 2012). The aligned FASTA datasets were converted to nexus format using Mesquite 3.61 (Maddison and Maddison 2019) and further analysed using MrBayes 3.2.6 (Ronquist et al. 2012) on XSEDE. Bayesian runs were computed independently twice with four MCMC chains for 10 million generations until the standard deviation of split frequencies fell below the 0.01 threshold. The convergence of runs was visually assessed using the trace function in Tracer 1.6 (Rambaut et al. 2013). The trees were visualized and annotated with TreeGraph 2 (Stöver and Müller 2010) and graphically improved in CorelDRAW X5 (Ottawa, Canada).

### Morphological observations

Based on field observations and photographs, we assigned basic general basidiomata morphology to the resulting clades. Since previous works have emphasized the importance of spore characters in this genus, we measured spore dimensions and amyloid reaction of five individuals (when available) per each operational taxonomic unit (OTU) defined in our *Dermoloma* phylogeny. Spores were observed under an Olympus BX43 microscope by Promicra 3-3CP camera. Spores were measured 20 or 30 times per sample using QuickPHOTO MICRO 3.2 software. Spore amyloidity was assessed in Melzer's reagent. Spore statistics were calculated with MS Excel and plotted using CorelDRAW X5 software.

## Results

### Phylogeny

In total, we analysed 119 *Dermoloma* samples. In our multi-locus analysis, they are represented each by ITS sequences (119 in total) and by 55 LSU sequences, 51 *rpb2* sequences and 17 *rpb1* sequences (Supplementary Table S1). The resulting alignment for the Tricholomataceae dataset resulted in 453 nrITS positions, 882 nrLSU positions, 449 *rpb1* positions and 699 *rpb2* positions. The *Dermoloma* dataset alignment

consisted of 568 nrITS positions, 805 nrLSU positions and identical *rpb1* and *rpb2* position numbers.

Almost all of the sequenced *Dermoloma* samples were placed in a large and well-supported monophyletic group within the family Tricholomataceae. This core *Dermoloma* clade is a sister to a clade containing the genus *Pseudotracheloma*, and a clade we referred to as ‘*Dermoloma*-like’ (Fig. 1). There is distinct clustering within the genus that corresponds to two clades classified at the subgeneric rank into the subgenus *Dermoloma* and the subgenus *Amylospora* subg. nov. (for formal designation of the new name, see below). The subgenus *Dermoloma* was identified by placement of the neotype sequence of the type species *Dermoloma cuneifolium* (Fr.) Singer ex Bon. Each subgenus consisted of one large core clade with tens of collections and a small residual clade with few samples. Both small residual clades were strongly supported on a long branch, and therefore, we propose further subdivision of subgenera into sections. Sections *Dermoloma* and *Conica* sect. nov. are placed in the subg. *Dermoloma* and sections *Atrobrunnea* Contu and *Nigrescentia* sect. nov. in the subgenus *Amylospora*. Two sequences putatively identified as *Dermoloma* (*Dermoloma*-like clade in Fig. 1) may represent an undescribed genus sister to *Pseudotracheloma*.

We recognised, in total, 31 OTUs within the genus *Dermoloma* that may represent potential species (Fig. 2). Fifteen OTUs are represented by a single collection. Section *Dermoloma* is represented by 11 OTUs, section *Conica* by two, section *Atrobrunnea* by 16 and section *Nigrescentia* by a single species *Dermoloma magicum* Arnolds. All OTUs strictly consisted of material from a single continent, either Europe or North America. Five OTUs of the section *Atrobrunnea* were North American, and a single representative of the section *Dermoloma* is a collection from Tennessee (USA) placed as a sister to *D. cuneifolium*. All other OTUs in our analysis originated from Europe.

Sequencing of 19 available type specimens from Europe and North America (Table 2) and authentic material of ‘*Dermoloma simulatum*’ nom. inval. resulted in 12 successful attempts. Unfortunately, the remaining samples yielded either contaminant or low-quality DNA due to old or poorly preserved specimens. Nine type sequences were represented in our phylogenetic tree (Fig. 2). Based on ex-type sequence position, we identified *D. cuneifolium*, *Dermoloma alexandri* Cons., *Dermoloma atrocinerum* (Pers.) P.D. Orton, *Dermoloma phaeopodium* P.D. Orton and *Dermoloma pusillum* Contu. Two collections clustered in our tree with the type of *D. hymenocephalum* (A.H. Sm.) Singer (Fig. 2), and since they are morphologically similar, we considered them as belonging to this species; the low BI support is probably due to short- and low-quality sequences of the type. *Dermoloma emilii-dlouhyi* Svrček is a synonym of *D. cuneifolium*,

and *Dermoloma pragense* f. *obscurum* Cons. & Contu is a synonym of *D. atrocinerum*. The ex-type sequence of *Dermoloma hygrophorus* also belongs to the genus *Dermoloma*, but it is not represented by a recent collection in our tree. Type sequencing excluded *Dermoloma intermedium* var. *coniferarum* Bon as a member of the genus; indeed, a 100% sequence similarity, based on a BLAST search, suggested this taxon corresponds to *Pseudolaccaria pachyphylla* (Fr.) Vizzini & Contu.

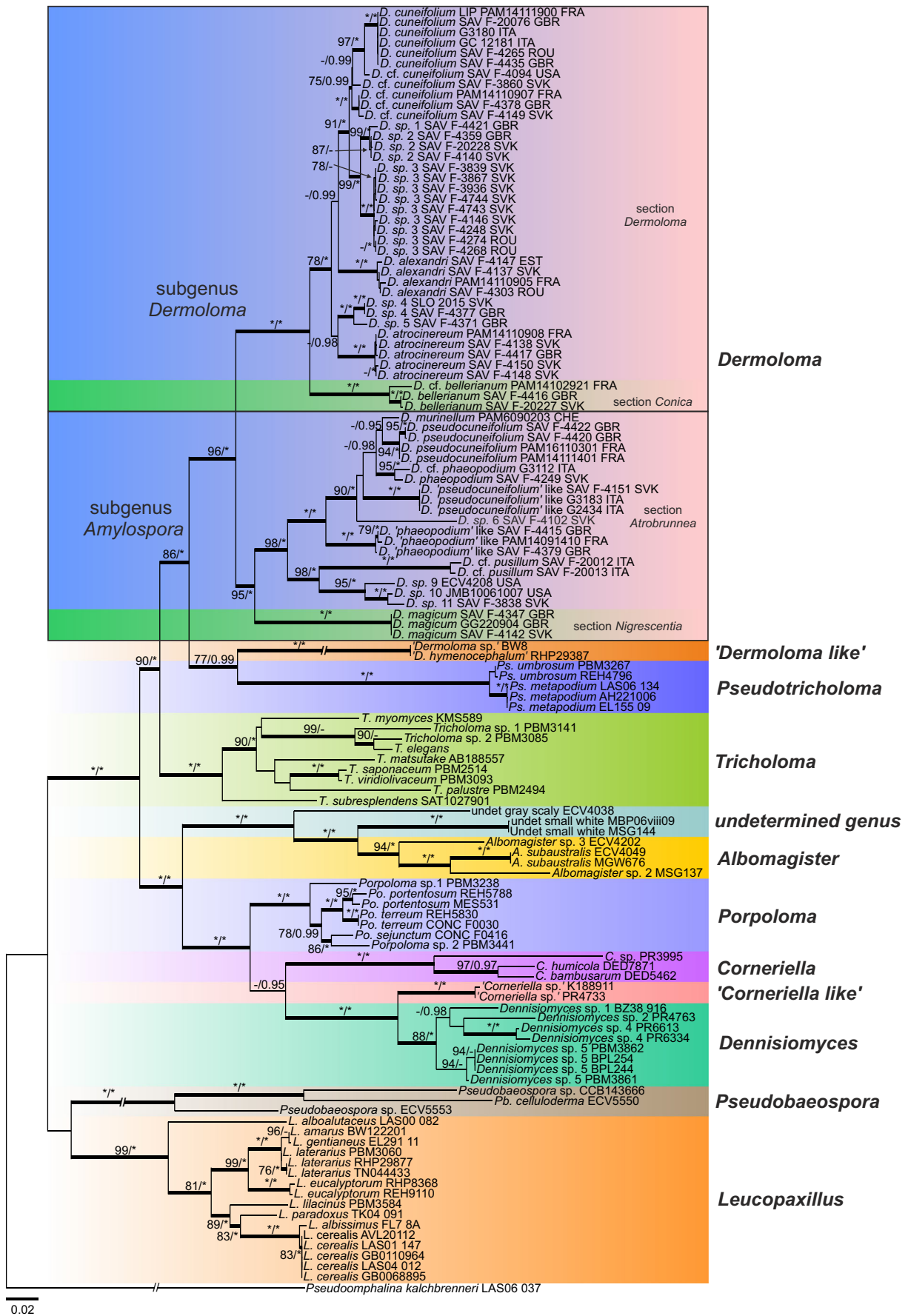
## Morphological observations

Our field observations were based on material collected by the authors that consisted of 92 specimens of the genus *Dermoloma* (more than 80% of all studied samples). Our observations confirmed the previous delimitation of the genus defined by Arnolds (1992): basidiomata with collybioid or tricholomatoid habit; adnate, sinuate or subdecurrent lamellae; farinaceous odour; and a white spore deposit. In addition, we observed only dull colours on basidiomata that combine grey, brown or white. Microscopic structure of all sequenced collections is always a hymeniderm or pruristratous hymeniderm, and the hyphae always bear clamp connections.

Two North American collections (TENN-F-029387, TENN-F-066899) identified putatively as *Dermoloma* but placed on a residual branch sister to *Pseudotracheloma* have similar morphology to *Dermoloma*. The basidiomata are fleshy and unstaining, lack a veil and resemble in stature *D. magicum*; the pale greyish colouration matches *Dermoloma bellerianum*; the pileipellis is a hymeniderm; the spores are amyloid, short-ellipsoid and white in deposit; and clamp connections are present. In addition to this unusual combination of characters, they can be distinguished from *Dermoloma* by an unpleasant rancid odour even when fresh, although the taste is farinaceous. Macrochemical reactions, viz. KOH and PDAB, are negative.

Infrageneric classification of the genus corresponded to two previously recognised groups: species with inamyloid spores are classified here in the subgenus *Dermoloma* (Fig. 3a) and species with amyloid spores in the subgenus *Amylospora* (Fig. 3b). These two groups are usually easily distinguishable in the field: the first has pilei usually with mainly grey colour and pale grey to almost white hymenium and stipe. The second group has either brown-grey pilei or darker grey or grey-brown hymenium and stipe.

Representatives of sect. *Conica* were distinguished from other inamyloid species of the core section by large basidiomata with conical pilei and very pale, almost white lamellae and stipe (Fig. 3c, d). The single species of sect. *Nigrescentia*, *D. magicum*, is distinguished from other species with amyloid spores (classified in sect. *Atrobrunnea*) by blackening basidiomata (Fig. 3e, f).





◀ **Fig. 1** Maximum likelihood (RAxML) phylogeny of Tricholomataceae inferred from nrITS, nrLSU, *rpb1* and *rpb2* loci. Maximum likelihood bootstrap support values greater than 70 and Bayesian posterior probabilities greater or equal to 0.95 are indicated at nodes. Bold lines represent branches supported by both ML and BI. Asterisks indicate ML = 100 or BI = 1.00

Morphological identification of species was traditionally based on spores. Therefore, we took special attention to spore observations and measured up to 5 collections (depending on material availability) per each OTU defined in our phylogenetic tree (Fig. 2). The majority of OTUs had spores 5–6.5  $\mu\text{m}$  long with a length-and-width ratio ( $Q$ ) of 1.25–1.50. Members of sect. *Dermoloma* showed little variability and fell within these ranges. Two OTUs of sect. *Conica* had narrower spores compared to members of sect. *Dermoloma* but showed no spore differences between each other. *Dermoloma magicum* of sect. *Nigrescentia* had large spores exceeding 7  $\mu\text{m}$  in length. There was only a single OTU of sect. *Atrobrunnea* with such large spores (*D. sp.* 10). More distinct differences in both length and shape ( $Q$  value) were observed among members of sect. *Atrobrunnea*. Our spore measurements support the identity of two US collections clustered with the ex-type sequence of *D. hymenocephalum* without a good BI support in our phylogenetic analysis (Fig. 2), probably due to low type sequence quality. Spores of these two collections are similar to the type, and we suggest that all three collections represent a single species.

Some taxa in this study were not identified by a sequence from a type or authentic material but by morphology. Our sequencing of the type species of sect. *Atrobrunnea*, *Dermoloma atrobrunneum* (Dennis) Bon (described from Trinidad), failed, but our morphological analyses confirmed the pileipellis structure and amyloid spores were typical for *Dermoloma pseudocuneifolium* and other members of this group. The conical pilei of *D. bellerianum* and blackening basidiomata of *D. magicum* defined these two species sufficiently. *Dermoloma murinellum* E. Horak was represented by a single collection from the type locality at high elevation in the Alps, and the spores matched our observations of the type specimen. We assigned the species name *D. pseudocuneifolium* to the largest supported clade of sect. *Atrobrunnea* that corresponded to a widely adopted concept of the species. Based on pileipellis and spore morphology, we confirmed that *Dermoloma fuscobrunneum* P.D. Orton, *D. intermedium* Bon and *Dermoloma longibasidiatum* Cons., Contu & Setti belonged to sect. *Dermoloma*, and *Dermoloma josserandii* Dennis & P.D. Orton and *D. pragense* Kubička belong to sect. *Atrobrunnea*, but their phylogenetic positions were not resolved (and these names were not represented in our tree). Our morphological study excluded *Dermoloma hybridum* (Kühner) Bon as a member of the genus *Dermoloma* because the type has a pileipellis of

repet cylindrical hyphae possibly corresponding to the genus *Tricholoma* Singer.

## Taxonomy

***Dermoloma*** (J. Lange) Singer ex Herink, Sborník Severočeského Musea 1: 62. 1958

≡ *Tricholoma stirps Dermoloma* J. Lange, Dansk. Bot. Ark. 8(3): 12. 1933

Type: *Dermoloma cuneifolium* (Fr.) Singer ex Bon

Description: Basidiomata agaricoid with well-developed central pileus and lamellate hymenophore. Pileus up to 75 mm wide, mainly convex and expanding to plain when mature, with pale or dark colours, combining white, brown and grey tints, sometimes distinctly hygrophanous, translucent-striate or not, surface usually smooth, glabrous, at times cracked when dry. Lamellae adnate to sinuate, at times weakly decurrent, concolourous or paler than pileus. Stipe usually equally long or longer than pileus diameter, up to 15 mm wide, central, cylindrical, sometimes narrowly fusiform or attenuated near the base, usually coloured like the pileus. Context fragile in pileus, white or pale, odour strongly farinaceous. Pileipellis a hymeniderm, pluristratous hymeniderm or transitional to an epithelium, composed of one or two rarely more layers of inflated, broadly clavate, sphaeropedunculate cells. Basidiospores broadly ellipsoid to ellipsoid, usually almost symmetrical, thin-walled, hyaline, white in deposit. Caulocystidia always present. Hyphae with clamp connections, hyphal terminations in pileipellis and stipitipellis with dark brown parietal or sometimes also incrusting pigments.

### ***Dermoloma*** subgenus ***Dermoloma***

Description: Basidiomata usually with dominating grey tints. Spores inamyloid

### ***Dermoloma*** subgenus ***Amylospora*** Adamčík, subgenus novus

Type: *Dermoloma phaeopodium* P.D. Orton

Mycobank No.: MB 837547

Diagnosis: Basidiomata usually with dominating brown tints. Spores amyloid

Etymology: The name refers to amyloid spores.

### ***Dermoloma*** section ***Dermoloma***

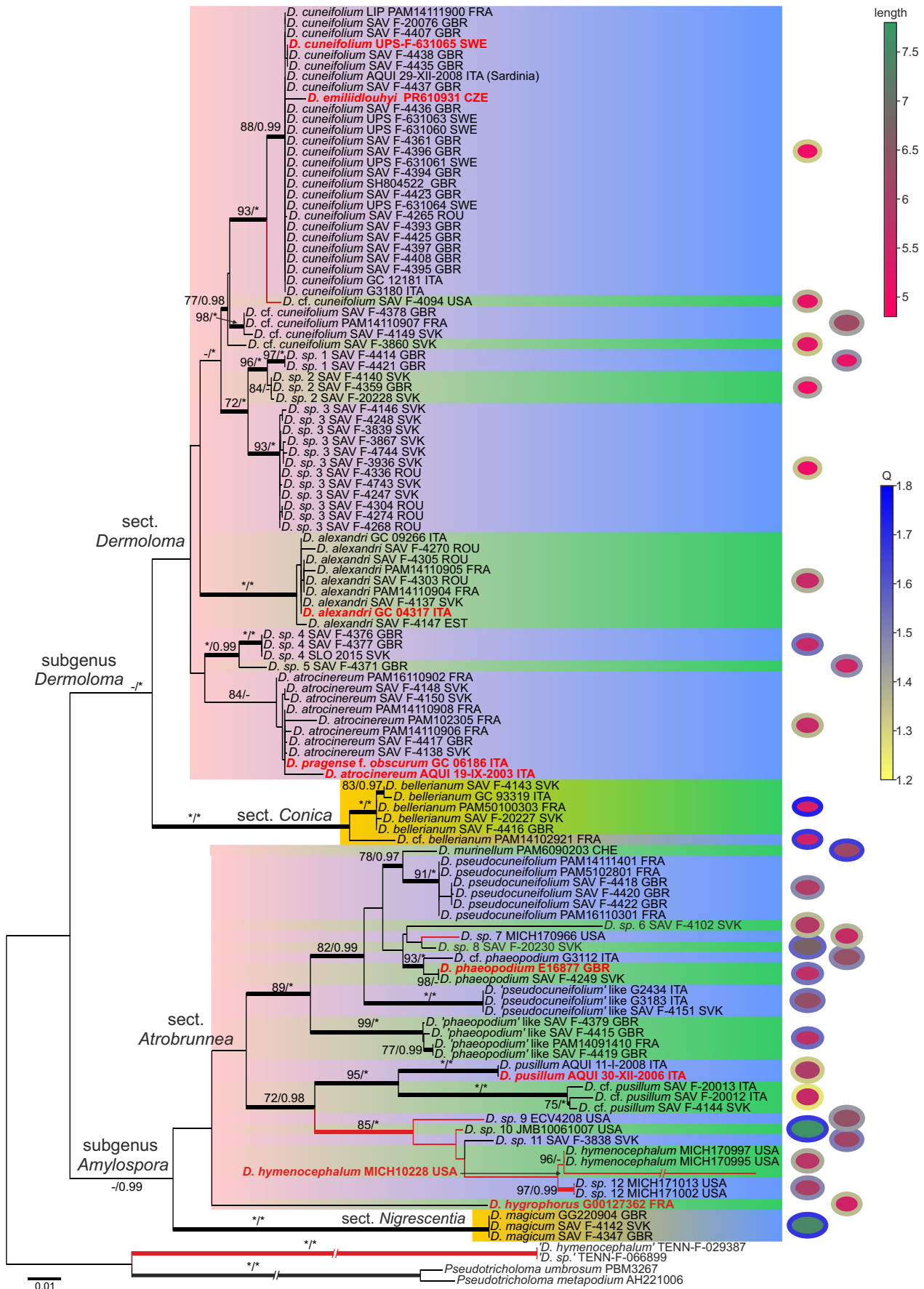
Description: Pileus convex and soon expanding plane. Spores broadly ellipsoid to ellipsoid,  $Q < 1.5$

### ***Dermoloma*** section ***Conica*** Adamčík, sectio nova

Type: *Dermoloma bellerianum* Bon

Mycobank No.: MB 837545

Diagnosis: Pileus conical, when old expanding plane and with distinct umbo. Spores ellipsoid to oblong,  $Q > 1.5$



◀ **Fig. 2** Maximum likelihood (RAxML) phylogeny of the genus *Dermoloma* inferred from nrITS, nrLSU, *rpb1* and *rpb2* DNA loci. Maximum likelihood bootstrap support values greater than 70 and Bayesian posterior probabilities greater or equal to 0.95 are indicated at nodes. Bold lines represent branches supported by both ML and BI. Asterisks indicate ML = 100 or BI = 1.00. Type specimens are indicated in red. American lineages are labelled in red and European ones in black. Spore pictures at the nodes show an amyloid reaction typical for each subgenus. Pictures of basidiomata show typical members of the sections: sect. *Dermoloma* is represented by *D. cuneifolium*, sect. *Conica* by *D. bellerianum*, sect. *Atrobrunnea* by *D. pseudocuneifolium* and sect. *Nigrescentia* by *D. magicum*. Diagrams to the right represent spores, and the size is proportional to the spore average calculated from the spore measurements from all the collections of the recognised lineages. The colours inside the ellipses correspond to spore length, and the colours of the outline correspond to the spore length/width ratio ( $Q$ ). Colour scales are shown to the right

Etymology: The name refers to conical pileus of the type species.

***Dermoloma* section *Atrobrunnea*** Contu, Boletim da Sociedade Broteriana 65: 80. 1992

Type: *Dermoloma atrobrunneum* (Dennis) Bon

Description: Context does not change colour when bruised.

***Dermoloma* section *Nigrescentia*** Adamčík, sectio nova

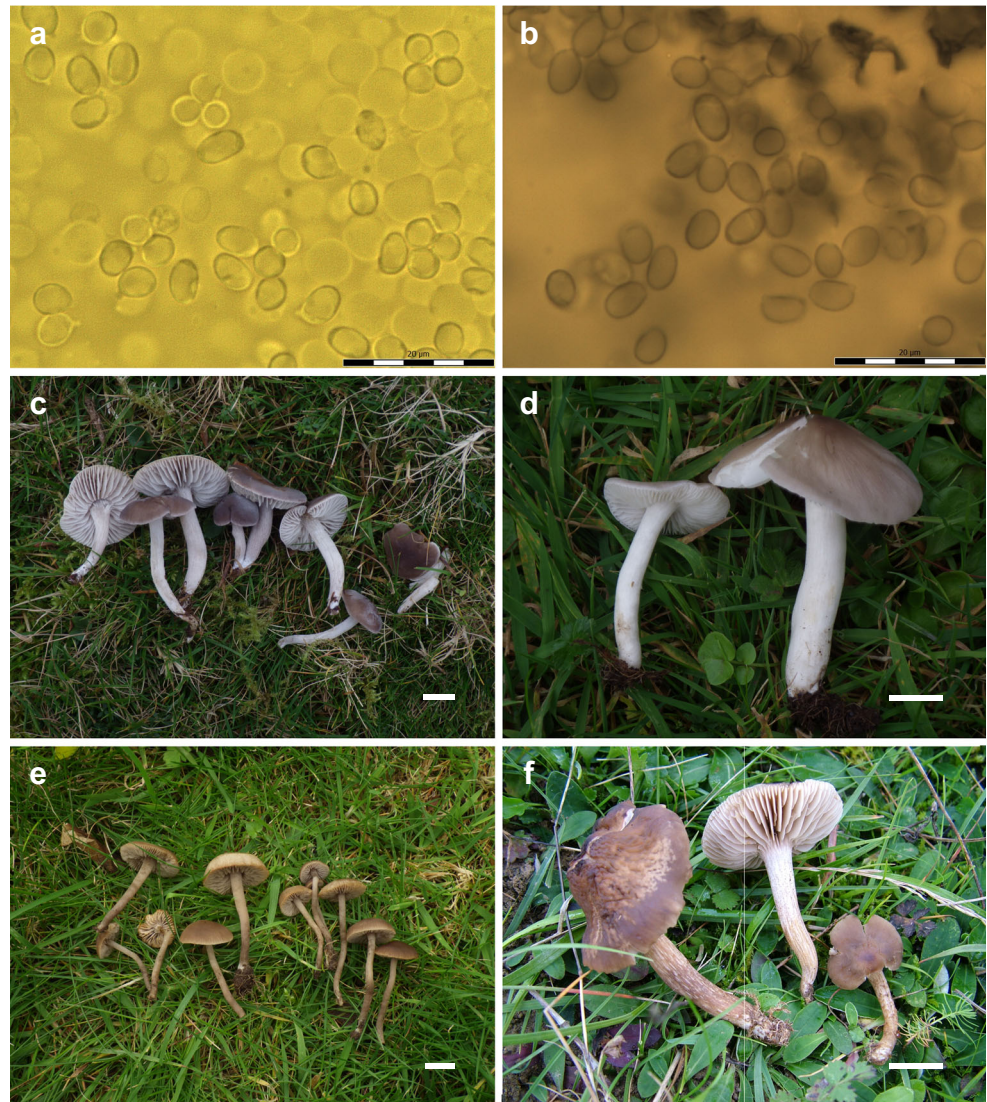
Type: *Dermoloma magicum* Arnolds

Mycobank No.: MB 837546.

Diagnosis: Context of basidiomata turns black when bruised.

Etymology: The name refers to colour change of basidiomata context when bruised.

**Fig. 3** Examples of *Dermoloma* morphology. **a** Inamyloid spores of subgenus *Dermoloma* (*D. cuneifolium*, SAV F-4423). **b** Amyloid spores of subgenus *Amylospora* (*D. pseudocuneifolium*, SAV F-4422). **c** Basidiomata of section *Dermoloma* (*D. cuneifolium*, SAV F-4436). **d** Basidiomata of section *Conica* (*D. bellerianum*, SAV F-4416). **e** Basidiomata of section *Atrobrunnea* (*D. pseudocuneifolium*, SAV F-4420). **f** Basidiomata of section *Nigrescentia* (*D. magicum*, SAV F-4242). Scale of 10 mm represents for basidiomata



## Discussion

### Phylogenetic placement of the genus

Our phylogenetic study confirmed that the genus *Dermoloma* is a member of the Tricholomataceae as resolved by the most recent phylogenetic study that includes the family (Sánchez-García and Matheny 2016). The latter study placed the genus *Pseudotracheloma* nested within samples identified as *Dermoloma*, because of one sample recognised in our study as *Dermoloma*-like genus (Fig. 1). The study of Sánchez-García and Matheny (2016) was the first showing the phylogenetic placement of the genus, but our study suggested surprisingly high diversity within the genus not expected by any previous study. These results are even more surprising because this genus with at least 23 species in Europe and probably with worldwide distribution has not been included in phylogenetic studies that focused on the Tricholomataceae (Sánchez-García and Matheny 2016; Hofstetter et al. 2014). Taxonomic identity of the genus *Dermoloma* in our study is resolved by the placement of the type species *D. cuneifolium*. The phylogenetic placement of *Dermoloma inconspicuum* in the family Agaricaceae (Kropp 2008) indicates that this species is one of a few that has yet to be combined in other genera, together with *D. hybridum* and *D. intermedium* var. *coniferarum*, which were excluded from *Dermoloma* in this study.

### Morphological delimitation of the genus and its lower rank taxa

Our study showed clear morphological and phylogenetic circumscription of the genus *Dermoloma*. Morphologically, it is defined by a combination of farinaceous odour and pluristratous hymeniderm pileipellis of inflated balloon-shaped elements. However, both characters are present also in multiple phylogenetic lineages of the Tricholomataceae and they should not serve as limits to define a group on above-family rank as proposed by Bon (1979), who defined the family Dermolomataceae based on a hymeniderm pileipellis structure typical for the genus *Dermoloma*. Genera included by Bon in this family occurred in different places of the Tricholomataceae tree (Sánchez-García and Matheny 2016), and a very similar pileipellis structure can be observed in very unrelated agarics, for example in the genus *Hodophilus* (Birkebak et al. 2016).

Taxa excluded in this study either have no distinct smell or have different pileipellis structures. *Dermoloma* belongs to a group of small brown mushrooms (SBMs), the popular term referring to a hyperdiverse morphological fungal group of polyphyletic origin and difficult morphological identification of species. In our experience, *Dermoloma* can resemble, in the field, some members of *Dennisiomyces*, *Pseudoporpoloma*, *Pseudotracheloma*, *Tricholoma* and possibly also species out of Tricholomataceae. Field identification of *Dermoloma* should

be supplemented with microscopical observations, and we recommend paying special attention to spore and pileipellis morphology.

It is worth to mention inconsistencies about spore amyloidity of some species in the literature. Even the concept of the type species *D. cuneifolium* was inconsistent. Some authors attributed amyloid spores to this species (Josserand 1958; Svrček 1966), but Orton (1980) disagreed with this interpretation and he thought that the species has inamyloid or sometimes weakly amyloid spores. In response, Arnolds (1992) designated a neotype for *D. cuneifolium* with inamyloid spores and Bon (1986) proposed the name *D. pseudocuneifolium* for *D. cuneifolium* in sense of Josserand (1958) (with amyloid spores). The confusions about spore amyloidity have not stopped afterwards. In our study, type specimens of *D. alexandri* and *D. pragensis* f. *obscurum* are placed in the section *Dermoloma* but original descriptions (Contu et al. 2008) report weakly amyloid spores. This weakly amyloid reaction is observed as a grey hue under certain focus in the light microscope, and it is usually seen in some members of the section *Dermoloma*. We performed a test placing lamellae fragments of *D. cuneifolium* and *D. alexandri*, reported as having non-amyloid and weakly amyloid spores respectively by Contu et al. (2008), but there was no distinguishable difference in the amyloid reaction. We therefore recognised as amyloid spores only those with strong reaction resulting to dark grey to black tints when spores are accumulated in mass or collapsed.

The species number in the subgenus *Dermoloma* accepted in the literature (Arnolds 1992, 1993; Contu et al. 2008; Vesterholt 2008, 2012) ranges between three to eight, whilst we recognised 12 species supported by our phylogeny (Fig. 2). Our morphological observations suggest that spore characters used in the literature to distinguish species within the subgenus *Dermoloma* are usually not reliable, e.g. *D. cuneifolium*, *D. alexandri* and *D. atrocinerum* have very similar spores (Fig. 2). Future taxonomic studies should include more observations of pileipellis, stiptipellis and lamellae edges, but also more detailed descriptions of macromorphological characters.

### Species diversity, distribution and ecology

A high number of singletons in our tree and apparent geographic limits of some species suggest that species diversity in Europe might be even higher than the 23 recognised OTUs. Based on the sequenced types and morphological characters, we assigned ten species names to the clades in our tree (Fig. 2). We did not study the type of *D. coryleti*, but based on the original description (Singer and Cléménçon 1971), it does not have a distinct farinaceous odour and the spores are inamyloid and much larger than any species of the section *Dermoloma* mentioned in this study. Therefore, it is likely that this species is not a member of the genus *Dermoloma*. Concluding from all our abovementioned observations, for

the remaining 17 unidentified OTUs in our tree, we have only available five species names that were not assigned to a phylogenetically identified OTU: *D. fuscobrunneum*, *D. intermedium*, *D. josserandii*, *D. longibasidium* and *D. pragense*. Some of them may represent synonyms. It means that a minimum of 12 species in Europe are so far undescribed. In addition, some species might have not been included in our study. To avoid nomenclatural confusions, a description of new European *Dermoloma* species will require not only type studies but detailed observations of morphological characters combining more characters than are so far used in the nomenclature.

Our study strongly suggests that North America has its authentic *Dermoloma* diversity different from Europe, because none of the OTUs studied was found in both, Europe and North America. The sampling of the genus in our tree seems to be even more insufficient for North America with only 6 recognised OTUs. Five OTUs are members of the section *Ato brunnea* together with *D. hymenoccephalum*. The single Tennessee (USA) sample in the tree placed in the section *Dermoloma* is probably the first published record of a species without amyloid spores in North America. There is an online record of a non-amyloid species identified as *D. cuneifolium* ([http://www.mushroomexpert.com/dermoloma\\_cuneifolium.html](http://www.mushroomexpert.com/dermoloma_cuneifolium.html)), but this record seems to be misidentified because it has amygdaliform spores that are much narrower than we observed within the genus *Dermoloma*. This online record is another evidence of insufficient and very rudimentary knowledge of the genus in North America.

Ecology of members of *Dermoloma* is equal to other members of the so-called CHEGD fungi, where this genus is included together with Clavariaceae, *Hygrocybe* (and other genera of Hygrophoraceae previously placed in this genus), *Entoloma* and Geoglossaceae (and some species of Leotiaceae previously placed in this family). In Europe, CHEGD fungi are typically found in grassland habitats, but in North America, they usually grow in forests associated with non-ectomycorrhizal trees (Adamčík et al. 2016; Lodge et al. 2014). It seems that more important than the presence or absence of trees is their affinity to undisturbed sites with a conservation value (Griffith et al. 2013). Their ecology may have a strong link to trophy that is so far unresolved. Analysis of C and N isotopes and  $^{13}\text{C}$  pulse label experiments and high natural abundance stable isotopes of  $^{15}\text{N}$  and low of  $^{13}\text{C}$  in basidiocarps suggest that they form unspecified biotrophic associations with plants (Halbwachs et al. 2018). They have a particular response to available nutrition changes linked to management practices when compared to other grassland fungi (Caboň et al., unpublished data). In our opinion, at least two other *Tricholomataceae* genera, *Pseudobaespora* and *Pseudotracheloma*, are associated with undisturbed traditionally managed grasslands and accompany *Dermoloma* species and should be included among other CHEGD fungi.

## Conclusion

Our results demonstrated that the real number of species in Europe and North America is higher than expected according to the literature, and the number of OTUs recognised in our phylogeny is much higher than the number of available published *Dermoloma* names. Before the description of new species, there is an urgent need to fix the morphological concept of already described species and to designate epitypes that will represent species in the phylogeny and provide reference sequences. The morphological arguments and characters used in the previous literature must be reconsidered and supplemented by new characters and more precise observations. The high number of OTUs represented by a single sample suggests that the diversity of the genus will be even higher with increasing sampling efforts from Europe, North America and elsewhere. There are 13 *Dermoloma* names from other continents, but the only available sequence from outside the USA and Europe corresponds to *D. inconspicuum* from Belize (type of the species is from Venezuela). This suggests that extra-European taxa need urgent revision and sequence data to specify their geographical distributions and morphological and genetic limits.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11557-020-01651-y>.

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**Authors' contributions** SA and MSG designed the study, contributed to the main conceptual ideas and wrote the paper with support from PBM. KA and MSG performed the DNA extractions, and SA and MSG edited the sequences. MSG, MC and SA designed the molecular part of the study and performed the molecular and phylogenetic analyses. MK, SJ and SA performed the morphological measurements. PBM, PAM and AV contributed to the data interpretations. AV, PAM, SA and SJ and contributed with collecting of European samples and macromorphological descriptions, and MSG and PBM provided the study with the material and sequences of North American species. PBM provided critical feedback to the study design and paper. All authors revised and approved the final manuscript.

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**Data availability** The sequences generated and/or analysed during the current study are available in the GenBank repository (<https://www.ncbi.nlm.nih.gov/genbank/>). All analysed specimens are deposited in public herbaria indicated in the [Supplementary table](#).

## Compliance with ethical standards

**Competing interests** The authors declare that they have no competing interests.

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