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MORPHOLOGICAL AND MOLECULAR CONFIRMATION FOR THE SOUTHERNMOST ITALIAN RECORD OF *LECCINUM CYANEOBASILEUCUM* VAR. *BRUNNEOGRISEOLUM* (*BOLETACEAE, BOLETALES*)

Abstract

The birch-loving bolete species Leccinum cyaneobasileucum var. brunneogriseolum is reported for the first time for Latium (central Italy) based on morphological, ecological and phylogenetic evidence. Various samples were collected in a bushland dominated by Betula pendula neighbouring a sulphureous bog. A complete macro- and micro-morphological description including environmental data is provided and accompanied by colour images of fresh basidiomes in habitat and line drawings of the main anatomical features. A single-gene molecular analyses based on DNA sequences of the single-copy nuclear gene glyceraldehyde 3-phosphate dehydrogenase (gapdh) has been produced to substantiate the taxonomic identification of Leccinum cyaneobasileucum var. brunneogriseolum and ascertain its phylogenetic placement. Comparisons with closely allied, phenotypically and molecularly similar taxa are also elucidated and discussed. To the best of our knowledge the present records represent the southernmost confirmed occurrence of this taxon in Italy. On account of molecular phylogenetic inference Leccinum subsect. Fumosa is recombined and upgraded to sectional rank.

Riassunto

La specie boletoide simbionte elettiva della betulla Leccinum cyaneobasileucum var. brunneogriseolum è segnalata per la prima volta dal Lazio (Italia centrale) sulla base di evidenza morfologica, ecologica e filogenetica. Varie raccolte sono state effettuate in un boschetto dominato da Betula pendula adiacente a una palude sulfurea. Si fornisce un'accurata descrizione dei caratteri macro- e micro-morfologici inclusiva dei dati ambientali, accompagnata da foto a colori di esemplari freschi in habitat e da disegni al tratto dei principali elementi anatomici. Un'analisi molecolare basata sul singolo marcatore deidrogenasi 3-fosfato gliceraldeide (gapdh) del rDNA nucleare è stata condotta per confermare l'identificazione tassonomica di Leccinum cyaneobasileucum var. brunneogriseolum ed accertarne la collocazione filogenetica. Le specie più affini sia sul piano fenotipico che molecolare vengono messe a confronto e discusse. Sulla scorta delle attuali conoscenze le nostre raccolte costituiscono il ritrovamento comprovato più meridionale di questo taxon per l'Italia. In base alla ricostruzione filogenetico-molecolare Leccinum subsez. Fumosa è ricombinata ed elevata al rango di sezione.

Key words: *Basidiomycota*, *Boletales*, ectomycorrhizal fungi, European bolete diversity, Latium, taxonomy.

Introduction

The genus *Leccinum* Gray, typified by *L. aurantiacum* (Bull.) Gray and classified in the family *Boletaceae,* is a long-standing boletoid genus whose establishment dates back to the third decade of the 19th century (Gray, 1821). For more than one hundred and twenty years this genus was practically not accepted by any author since it was considered a superfluous synonym of *Boletus* Fr., having been originally conceived as a broad heterogeneous assemblage of distantly related boletoid species which have later been accommodated in separate genera within the *Boletaceae*, *Gyroporaceae* and *Suillaceae* (Šutara, 1982).

A modern delimitation of *Leccinum* was reached only with the emendation by Snell (1942). Snell's redefinition of the generic limits of *Leccinum* marked the starting point of its current use and was followed by nearly all of the specialists worldwide in the subsequent decades (Watling, 1961, 1968; Smith *et al*., 1966, 1967, 1968; Horak, 1968; Smith & Thiers, 1971; Pilát & Dermek, 1974; Engel *et al*., 1978, 1983; Pegler & Young, 1981; Singer, 1986; Šutara, 1989; Korhonen, 1995; Lannoy & Estadès, 1995; Redeuilh, 1995; Hahn, 1997; Den Bakker, 2000; Cazzoli, 2002; Halling & Mueller, 2003; Muñoz, 2005; Fu *et al*., 2006; Ortiz-Santana *et al*., 2007). In Europe, after earlier works focused on the taxonomy and nomenclature of *Leccinum* (Berteaux, 1961; Watling, 1961, 1968; Blum, 1967a, b, 1970; Pilát & Dermek, 1974; Engel *et al*., 1978, 1983; Redeuilh, 1980; Bon & Van Haluwyn, 1981; Bon, 1984; Alessio, 1985; Šutara, 1989; Dörfelt & Berg, 1990), the genus was extensively investigated at the beginning of '90s by the French boletologists G. Lannoy and A. Estadès in a series of contributions (Lannoy & Estadès, 1991a, b, 1993, 1994) which culminated with the publication of a monographic work (Lannoy & Estadès, 1995), resulting in a steadily increase of the overall number of taxa at both the specific (up to some 40 species) and intraspecific ranks. Yet, many *Leccinum* species, varieties and forms described by the French authors were closely related to one another and difficult to identify. Reliance on color variation coupled with the occurrence of albinotic or simply discolored forms led to further confusion in species determination.

It was not until the early 2000s that a modern approach to the study of the taxonomic limits of the species in *Leccinum* was critically carried out by means of molecular phylogenetic techniques. Preliminary DNA-based accounts of European *Leccinum* were proposed by Binder (1999) and Binder & Besl (2000). The most comprehensive molecularly-based research on European species, however, was undertaken a few years later by Den Bakker *et al.* (2004a, b, 2007), Den Bakker & Noordeloos (2005) and Den Bakker (2006), who primarily tried to determine the real number of *Leccinum* species for the European mycota, and aimed also at clarify misleading species concepts as encountered in earlier taxonomic works. Based on the amplification of three loci (ITS, 28S and *gapdh*), they explored the diversity of the genus and reduced the number of European *Leccinum* to only 16 species. Their results, although considered by some authors too much conservative and in need of further testing and refinements to be relied on a finer phylogenetic resolution, established new criteria for assessing entities in *Leccinum*, heading to a clearer definition of their reciprocal taxonomic boundaries.

The outcomes achieved by Binder (1999), Binder & Besl (2000) and Den Bakker & Noordeloos (2005), reinforced by those obtained subsequently with a more inclusive sampling (Binder & Hibbett, 2006; Nuhn *et al*., 2013; Wu *et al*., 2014, 2016b), emphasized the polyphyletic nature of *Leccinum* as previously delimited by means of morphological attributes. A number of novel genera have therefore recently been erected, namely *Leccinellum* Bresinsky & Manfr. Binder, *Hemileccinum* Šutara, *Harrya* Halling, Nuhn & Osmundson, *Australopilus* Halling & Fechner, *Sutorius* Halling, Nuhn & Fechner and *Rugiboletus* G. Wu & Zhu L. Yang (Bresinsky & Besl, 2003; Šutara, 2008; Halling *et al*., 2012a, b; Wu *et al*., 2016a) to accommodate several unrelated species formerly placed in *Leccinum* s. l. Furthermore, molecular phylogenetic analyses placed *Leccinum* s. str. in the subfamily *Leccinoideae* and also highlighted its strict correlation with the pileate-stipitate *Leccinellum* and with the sequestrate hypogeous genera *Octaviania* Vittad., *Chamonixia* Roll., *Rossbeevera* T. Lebel & Orihara and *Turmalinea* Orihara & N. Maekawa (Bruns *et al*., 1998; Den Bakker & Noordeloos, 2005; Binder & Hibbett, 2006; Lebel *et al*., 2012; Orihara *et al*., 2012, 2016; Wu *et al.*, 2014, 2016b). However, as opposed to the current trend of proliferation of new genera, Kuo & Ortiz-Santana (2020) have most recently adopted a conservative approach to the systematic of the *Boletaceae*, and based on a purported paraphyly of the representatives of the core leccinoid clade as previously suggested by Den Bakker & Noordeloos (2005), Lebel *et al.* (2012) and Wu *et al*. (2014), they have collapsed all aforementioned genera into a very broadly circumscribed genus *Leccinum*.

Aside from the alternative systematic re-arrangement proposed by the two American authors, the most reliable morphological key features shared by the species of the genus *Leccinum* s. str. in its current, narrower circumscription include: medium to medium-large sized basidiomes, slender stipe which is usually much longer than the pileus diameter and typically ornamented lengthwise by prominent squamules or scabrosities tending to darken, whitish gray-cream to brownish hymenophore consisting of ventricose and extremely thin tubes that are longer than pileus context thickness and with very small, roundish pores, whitish context, large (rarely shorter than 15 µm), fusiform, smooth-walled basidiospores, pileipellis a trichoderm or cutis of interwoven filamentous hyphae sometimes intermixed with cylindrocysts, bilaterally divergent trama of the "*Boletus*-type", lateral stipe stratum of the "*leccinoid*" type, absence of pigments of the pulvinic acid derivatives and a natural distribution mostly throughout the temperate and boreal zones of the northern hemisphere (Binder & Besl, 2000; Den Bakker & Noordeloos, 2005; Šutara, 2005; Watling, 2008; Bessette *et al*., 2016; Wu *et al*., 2016b). Accordingly, *Leccinum* appears to be monophyletic only when restricted to the species traditionally belonging within sect. *Leccinum* (species characterized by the cuticle overhanging pileus margin and usually irregularly disrupted in appendiculate flaps, pileus mostly with reddish tones, context discoloring pinkish vinaceous and/or directly turning grayish violet and finally blackening, mean Q-value of basidiospores usually > 3.0 and association with both conifers and broadleaved trees), sect. *Scabra* A.H. Smith, Thiers & Watling (species characterized by the pileus margin entire, non-reddish pileus surface, context unchangeable or at most bruising pinkish vinaceous and an obligate association with *Betula* spp. or perhaps exceptionally also with *Salix* spp.) and sect. *Scabra* subsect. *Fumosa* A.H. Smith, Thiers & Watling (species characterized by the pileus margin entire, non-reddish pileus surface, context discoloring pinkish vinaceous and/or directly turning grayish to blackish, rarely unchanging and an obligate association with *Populus*) (Binder & Besl, 2000; Den Bakker & Noordeloos, 2005; Binder & Hibbett, 2006; Nuhn *et al*., 2013; Wu *et al*., 2016b). From an ecological viewpoint *Leccinum* comprises exclusively obligate ectomycorrhizal (ECM) fungi with several species being invariably restricted to single host genera, hence their geographic distribution roughly overlap that of their elective hosts. For example, species of sect. *Scabra* form ECM relationships exclusively with plants of the genus *Betula* and consequently they can be found only throughout the natural range of these companion plants, indicating a strong specialization of this section towards birches. Host specificity is important in understanding the biological affinities and ecological requirements of *Leccinum* species with respect to the potential associated vegetation and the key role they play in the plant communities of the temperate and boreal ecosystems where they commonly co-occur (Den Bakker *et al*., 2004a).

In the present communication we report on the first confirmed record of *L. cyaneobasileucum* var. *brunneogriseolum* in Latium (Central Italy) on account of morphological, ecological and molecular phylogenetic inference. Furthermore, the studied collections currently represent the southernmost occurrence of the geographic distribution range of *L. cyaneobasileucum* var. *brunneogriseolum* in Italy.

Materials and methods

Collection sites and sampling

In November 2019 fresh material was repeatedly collected at a single site in Latium, central Italy. Specimens of the collections examined in this study were deposited in the first author's private herbarium (MG) and one of these samples was duplicated in the fungal herbarium of Giampaolo Simonini (GS).

Herbarium numbers are cited for all collections from which morphological features were examined. Author citations follow the Index Fungorum - Authors of Fungal Names (www.indexfungorum.org/authorsoffungalnames.htm).

Morphological studies

Macroscopic descriptions, macro-chemical reactions (30% NH_4OH, 30% KOH, $FeSO_4$) and ecological information, such as habitat notations, time of fruiting and associated plant communities accompanied the detailed field notes of the fresh basidiomes. In the field, latitude, longitude, and elevation were determined with a Global Positioning System (GPS) receiver. Colors were recorded under daylight and described in general terms only. Photographs of collections were taken in the natural habitat using a Nikon D3100 camera. Microscopic anatomical features were observed and recorded from revived dried material; sections were rehydrated either in water, 5% potassium hydroxide (KOH) or in anionic solution saturated with Congo red. All anatomical structures were measured from preparations in anionic Congo red. Colours and pigments were described after examination in water and 5% KOH. Measurements were made at 1000× using a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Basidiospores were measured directly from the hymenophore of mature basidiomes, dimensions are given as (minimum) average ± standard deviation (maximum), Q = length/width ratio with the extreme values in parentheses, Qm = average quotient (length/width ratio) ± standard deviation and average spore volume was approximated as a rotation ellipsoid [$V = (\pi.L.W^2)/6 \pm$ standard deviation]. The notation [n/m/p] indicates that measurements were made on "n" randomly selected basidiospores from "m" basidiomes of "p" collections. The width of each basidium was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Radial and/or vertical sections of the pileipellis were taken midway between the center and margin of the pileus. Sections of the stipitipellis were taken from the upper part along the longitudinal axis of the stipe. Metachromatic, cyanophilic, and iodine reactions were tested by staining the basidiospores in Brilliant Cresyl blue, Cotton blue, and Melzer's reagent, respectively. Line drawings of microstructures were traced in free hand based on digital photomicrographs of rehydrated material.

DNA extraction, PCR amplification and DNA sequencing

DNA extraction and PCR amplification were performed from dried basidiomata (**Table 1**) as described by Vizzini *et al*. (2020). Primers ITS1F and ITS4 (White *et al*., 1990; Gardes & Bruns, 1993) were used for the ITS region. Primers gpd_g_f (gpd general forward) and gpd_g_r (gpd general reverse) (Den Bakker *et al*., 2004b) were used for the *gapdh* rDNA. The PCR products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega) following manufacturer's instructions and positive reactions sequenced forward and reverse by MACROGEN Inc. (Seoul, Republic of Korea).

Sequence alignment, data set assembly and phylogenetic analyses

For the single-locus dataset of *Leccinum*, one *gapdh* sequence from MG789 (MZ041658) and two ITS sequences from MG789 (MZ041710) and MG797 (MZ041711) were newly generated and deposited in NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The chromatograms were examined and manually edited for accuracy and the *gapdh* sequence obtained was checked and assembled using MEGA-X (Kumar *et al*., 2018). The newly acquired *gapdh* sequence was compared to those available in GenBank by using the Blastn algorithm (Altschul *et al*., 1990) and then combined with homologous selected sequences of vouchered specimens from previous studies (**Table 1**).

The alignment was generated for the *gapdh* dataset and manually adjusted with MEGA-X (Kumar *et al*., 2018). *Leccinellum* sequences (*Leccinum carpini* (R. Schulz) M.M. Moser ex D.A. Reid and *L. crocipodium* (Letellier) Watling) and *Leccinum talamancae* Halling, L.D. Gómez & Lannoy were chosen as the outgroup based on the phylogeny in Den Bakker *et al.* (2007). Phylogenetic hypotheses were constructed with Bayesian inference (BI) and Maximum likelihood (ML) criteria. The partitioned BI was performed with MrBayes v. 3.2.7a (Ronquist *et al*., 2012) with one cold and three incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 M generations, under the selected evolutionary models for each unlinked partition. Two simultaneous runs were performed independently. Trees were sampled every 1000 generations, resulting in sampling of 10001 trees per single run with the first 2500 trees (25%) discarded as burn-in. For the remaining trees of the two independent runs, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). Partitioned ML analyses was performed using RAxML v. 7.3.2 (Stamatakis, 2006) with 1000 bootstrap replicates (Felsenstein, 1985) and the GTRGAMMA model of sequence evolution. Support values from bootstrapping runs (MLB) were mapped on the best ML tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. BPP values ≥ 0.95 and MLB values ≥ 70%, are reported in the resulting tree (**Fig. 1**). Branch lengths were estimated as mean values over the sampled trees.

Results

Molecular analyses

Both Bayesian and Maximum Likelihood analyses produced comparable topologies and therefore only Bayesian trees with BPP and MLB values are shown (**Fig. 1**). The single-locus dataset (*gapdh*) of *Leccinum* consisted of 78 accessions and 1053 nucleotide sites. In the individual *gapdh* analyses three major monophyletic subclades were recovered within the genus *Leccinum*, corresponding to sections *Fumosa* (see discussion), *Leccinum* and *Scabra*, respectively. In the *Scabra* clade all European and North American accessions of *L. variicolor* grouped together with a strong statistical support (BPP = 1; MLB = 100%). Sister to the *L. variicolor* lineage was a large clade including accessions of *L. rotundifoliae*, *L. schistophilum*, *L. snellii*, *L. holopus*, *L. cyaneobasileucum* var. *brunneogriseolum* and *L. scabrum*/*L. melaneum*, all of these species being consistently well-supported in their own terminal clades with the exception of *L. melaneum* whose sequences fell within the *L. scabrum* clade. The newly generated *gapdh* sequence derived from MG789 nested within a highly supported *Leccinum cyaneobasileucum* var. *brunneogriseolum* lineage (BPP = 1; MLB = 99%) along with three other contaxic sequences from France and Holland. This lineage is recovered as sister to *L. holopus* although with no statistical support (BPP = 0.53; MLB = 58%).

Table 1. Details of specimens used in the phylogenetic analyses. The newly obtained sequence is in bold.

Voucher specimen	Species (original name from GenBank)	Geographic origin	GenBank accession number *gapdh*	Reference
Arora 00-293	*L. arbuticola*	Boonville, Mendocino County, CA, USA	AY538791	Den Bakker *et al.* 2004b
27-8-84/3	*L. atrostipitatum*	Canada, Quebec, Nouveau Quebec	AY538803	Den Bakker *et al.* 2004b
RW 1656	*L. aurantiacum*	France, Ardennes, Sommauthe/ Beaumonten-Argonne	AY538795	Den Bakker *et al.* 2004b
hdb 093	*L. aurantiacum*	Norway, Sogn og Fjordane, Sogndal	AY538817	Den Bakker *et al.* 2004b
hdb 039	*L. brunneogriseolum*	Holland, Friesland, Schiermonnikoog	AY538806	Den Bakker *et al.* 2004b

van Brummelen isolate 189	*L. brunneogriseolum*	France, Orne, Forêt de Bellême	AY853499	Den Bakker *et al.* 2007
men 93201	*L. brunneogriseolum*	Holland, Gelderland, Ede, Ginkelse Zand	AY853500	Den Bakker *et al.* 2007
Redeuilh 2270 P	*L. callitrichum* (HOLOTYPE)	France, Lozère, Aumont- Aubrac	AY538818	Den Bakker *et al.* 2004b
hdb 065	*L. carpini*	Holland, Breukelen, Utrecht	AY538785	Den Bakker *et al.* 2004b
RW 1659	*L. crocipodium* (EPITYPE)	France, Ardennes, Sommauthe/ Beaumont-en-Argonne	AY538784	Den Bakker *et al.* 2004b
Halling 6914	*L.* cf. *snellii*	USA, NC, Swain County, Indian Creek	AY538811	Den Bakker *et al.* 2004b
Halling 4472	*L.* cf. *snellii*	USA, NY, Hamilton County, Raquette Lake,	AY538812	Den Bakker *et al.* 2004b
MG789	***L. cyaneobasileucum* var. *brunneogriseolum***	**Italy, Latium, Macchiagrande di Manziana, Solfatara**	**MZ041658**	**This study**
wt 01	*L. duriusculum*	Holland, Zuid Holland, Wassenaar	AY538787	Den Bakker *et al.* 2004b
hdb 307	*L. holopus*	Canada, Ontario, Haliburton	AY853505	Den Bakker *et al.* 2007
hdb 329	*L. holopus*	Canada, Onatrio, Manitoulin Island	AY538808	Den Bakker *et al.* 2004b
hdb 040	*L. holopus*	Holland, Zuid Holland, Nieuwkoop	AY538807	Den Bakker *et al.* 2004b
Estadès 9109251	*L. holopus* (HOLOTYPE *L. nucatum*)	France, Isère, Massif de l'Obiou	AY853502	Den Bakker *et al.* 2007
Arora 00-54	*L. holopus*	Canada,Yukon Territory, 30 mi north of Dawson City	AY853503	Den Bakker *et al.* 2007
J&N 8326	*L. holopus*	Greenland, Sisimiut	AY853504	Den Bakker *et al.* 2007
hdb 320	*L. insigne*	Canada, Ontario, Manitoulin Island	AY538822	Den Bakker *et al.* 2004b
LG 464	*L. manzanitae*	USA, CA, Santa Cruz Island	AY538789	Den Bakker *et al.* 2004b
ecv 2404	*L. manzanitae*	USA, California	AY538790	Den Bakker *et al.* 2004b
hdb 033	*L. melaneum*	Holland, Noord Holland, AWD	AY853517	Den Bakker *et al.* 2007
hdb 047	*L. melaneum*	Holland, Drenthe, Gieten, Boekweitveentje	AY853522	Den Bakker *et al.* 2007
Halling 8325	*L. monticola*	Costa Rica, San José, Dota, Cerro de la Muerte	AY538820	Den Bakker *et al.* 2004b

Halling 8288	*L. monticola* (HOLOTYPE)	Costa Rica, San José, Dota, Cerro de la Muerte	AY538788	Den Bakker *et al.* 2004b
Redeuilh 4676 P	*L. nigellum* (HOLOTYPE)	France, Vibraye	AY538815	Den Bakker *et al.* 2004b
MK 11145	*L. palustre* (HOLOTYPE)	Finland, Uusimaa, Vantaa, Veromies	AY538809	Den Bakker *et al.* 2004b
MEN 2084	*L. piceinum*	Austria, Lienz, Obertiliach	AY538794	Den Bakker *et al.* 2004b
van Brummelen 2	*L. populinum*	France, Orne, Ige	AY538823	Den Bakker *et al.* 2004b
A.E. Hills 2001219	*L. populinum*	England, Berkshire, Windsor Great Park	AY538819	Den Bakker *et al.* 2004b
MK 11850	*L. populinum* (HOLOTYPE)	Finland, Nylandia, Vantaa	AY538797	Den Bakker *et al.* 2004b
van Brummelen 3	*L. quercinum*	France, Doubs, Forêt de Cessey	AY538796	Den Bakker *et al.* 2004b
hdb 102	*L. quercinum*	Holland, Drenthe, Roden	AY538816	Den Bakker *et al.* 2004b
MK 11452	*L. roseotinctum*	Finland, Lappi, Enontekio Kilpisjarvi	AY538802	Den Bakker *et al.* 2004b
hdb 016	*L. rotundifoliae*	Sweden, Jämtland, Borgsjö	AY853508	Den Bakker *et al.* 2007
hdb 086	*L. rotundifoliae*	Norway, Jotunheimen	AY853507	Den Bakker *et al.* 2007
Estadès 891024/3	*L. rotundifoliae* (HOLOTYPE *L. pulchrum*)	France, Isère, Massif de Belledonne, Prabert	AY853510	Den Bakker *et al.* 2007
Estadès 8909284 bisA	*L. rotundifoliae* (HOLOTYPE *L. pulchrum f. fuscodiscum*)	France, Isère, Massif de Belledonne, Les Seiglières	AY853509	Den Bakker *et al.* 2007
J&N 8263	*L. rotundifoliae*	Greenland, Kellyville	AY853506	Den Bakker *et al.* 2007
hdb 315	*L. scabrum*	Canada, Ontario, Manitoulin Island	AY853514	Den Bakker *et al.* 2007
hdb 328	*L. scabrum*	Canada, Ontario, Manitoulin Island	AY853512	Den Bakker *et al.* 2007
hdb 048	*L. scabrum*	Holland, Drenthe, Hoogeveen	AY538813	Den Bakker *et al.* 2004b
hdb 316	*L. scabrum*	Canada, Ontario, Manitoulin Island	AY853511	Den Bakker *et al.* 2007
hdb 301	*L. scabrum*	Canada, Ontario, Midhurst	AY538814	Den Bakker *et al.* 2004b
van Brummelen isolate 186	*L. scabrum* (EPITYPE)	France, Guyancourt, Versaille	AY853520	Den Bakker *et al.* 2007
Arora 00-218	*L. scabrum*	USA, CT	AY853513	Den Bakker *et al.* 2007
hdb 231	*L. scabrum*	UK, Invernesshire, Insh Marshes	AY853515	Den Bakker *et al.* 2007

hdb 022	*L. scabrum*	Sweden, Jämtland, Borgsjö	AY853521	Den Bakker *et al.* 2007
hdb 080	*L. scabrum*	Sweden, Jämtland, Kall	AY853516	Den Bakker *et al.* 2007
hdb 095	*L. scabrum*	Norway, Sogn og Fjordane, Sognedallfjora	AY853523	Den Bakker *et al.* 2007
van Brummelen isolate 188	*L. scabrum*	France, Orne, Forêt de Belleme	AY853525	Den Bakker *et al.* 2007
hdb 087	*L. scabrum*	Norway, Sogn og Fjordane, Sognedallfjora	AY853519	Den Bakker *et al.* 2007
hdb 239	*L. scabrum*	UK, Invernesshire, Aberarder Forest	AY853524	Den Bakker *et al.* 2007
hdb 121	*L. schistophilum*	Holland, Noord Holland, AWD	AY853526	Den Bakker *et al.* 2007
van Brummelen 4	*L. schistophilum*	France, Orne, Forêt Dominial du Perche	AY538810	Den Bakker *et al.* 2004b
A.E. Hills isolate 191	*L. schistophilum*	UK, Gloucestershire	AY853527	Den Bakker *et al.* 2007
Smith 72119	*L. snellii* (HOLOTYPE)	USA, MI, Chippewa County, Emerson	AY853528	Den Bakker *et al.* 2007
hdb 327	*L. snellii*	Canada, Ontario, Manitoulin Island	AY538805	Den Bakker *et al.* 2004b
hdb 317	*Leccinum* sp.4	Canada, Ontario, Manitoulin Island	AY538821	Den Bakker *et al.* 2004b
Arora 00-53	*Leccinum* sp.3	Canada, Yukon Territory, Along Dempster Highway	AY538824	Den Bakker *et al.* 2004b
Halling 8001	*L. talamancae*	Costa Rica, San José, Dota, San Gerardo	AY538783	Den Bakker *et al.* 2004b
hdb 330	*L. uliginosum*	Canada, Ontario, Whitefish Falls	AY538786	Den Bakker *et al.* 2004b
hdb 007	*L. variicolor*	Sweden, Jämtland, Östavall	AY853534	Den Bakker *et al.* 2007
hdb 051	*L. variicolor*	Holland, Erica, Oosterse bos	AY538804	Den Bakker *et al.* 2004b
hdb 011	*L. variicolor*	Sweden, Jämtland, Östavall	AY853533	Den Bakker *et al.* 2007
hdb 078	*L. variicolor*	Sweden, Jämtland, Kall	AY853530	Den Bakker *et al.* 2007
hdb 090	*L. variicolor*	Norway, Sogn og Fjordane, Sogndal	AY853536	Den Bakker *et al.* 2007
hdb 139	*L. variicolor*	Holland, Overijsel, Italiaanse Meertjes	AY853532	Den Bakker *et al.* 2007
MK 10977	*L. variicolor*	Finland, Perä-Pohjanmaa, Rovaniemi	AY853529	Den Bakker *et al.* 2007

hdb 226	*L. variicolor*	UK, Invernessshire, Curr Wood	AY853531	Den Bakker *et al.* 2007
J&N 8328	*L. variicolor*	Greenland, Sisimiut	AY853535	Den Bakker *et al.* 2007
hdb 285	*L. versipelle*	Holland, Gelderland, Leusderheide	AY538799	Den Bakker *et al.* 2004b
hdb 070	*L. versipelle* (NEOTYPE)	Sweden, Jämtland, Kall	AY538801	Den Bakker *et al.* 2004b
OF 64036	*L. versipelle*	Norway, Sogn og Fjordane, Lærdal	AY538798	Den Bakker *et al.* 2004b
hdb 092	*L. vulpinum*	Norway, Sogn og Fjordane, Sogndal	AY538792	Den Bakker *et al.* 2004b
hdb 415	*L. vulpinum*	Canada, Ontario, Manitoulin Island	AY538793	Den Bakker *et al.* 2004b

TAXONOMY

Leccinum cyaneobasileucum var. ***brunneogriseolum*** (Lannoy & Estadès) Lannoy & Estadès, Bulletin de la Société Mycologique de France 123 (1): 45 (2008) ("2007")

MYCOBANK MB 532799

≡ *Leccinum brunneogriseolum* Lannoy & Estadès, Documents Mycologiques 21 (82): 1 (1991) (basionym)

≡ *Leccinum brunneogriseolum* var. *pubescentium* Lannoy & Estadès, Documents Mycologiques 21 (82): 2 (1991)

≡ *Leccinum brunneogriseolum* f. *chlorinum* Lannoy & Estadès, Documents Mycologiques 23 (89): 65 (1993)

≡ *Leccinum cyaneobasileucum* var. *brunneogriseolum* f. *chlorinum* (Lannoy & Estadès) Klofac, Österreichische Zeitschrift für Pilzkunde 25: 2 (2016)

≡ *Leccinum cyaneobasileucum* var. *brunneogriseolum* f. *pubescentium* (Lannoy & Estadès) Klofac, Österreichische Zeitschrift für Pilzkunde 25: 2 (2016)

= *Leccinum cyaneobasileucum* Lannoy & Estadès, Documents Mycologiques 21 (81): 23 (1991)

= *Leccinum cyaneobasileucum* var. *pubescentium* (Lannoy & Estadès) Blanco-Dios, Index Fungorum 383: 1 (2018)

– *Leccinum brunneogriseolum* var. *cyaneobasileucum* (Lannoy et Estadès) Lannoy et Estadès, Bulletin de la Société Mycologique de France 122 (4): 373 (2006) (nom. inval.)

? – *Krombholzia scabra* var. *coloratipes* Singer, Revue de Mycologie 5 (1): 12 (1940) s. auct. p.p. (nom. inval. Art. 39.1, no Latin diagnosis)

? – *Krombholzia scabra* var. *coloratipes* Singer, Annales Mycologici 40 (1-2): 36 (1942) s. auct. p.p. (nom. inval., Art. 39.1, no Latin diagnosis)

? – *Leccinum scabrum* f. *coloratipes* Singer, Die Pilze Mitteleuropas, Band 6. Die Röhrlinge. Teil 2. Die Boletoideaea und Strobilomycetaceae: 95 (1966) ("1967") (nom. inval.)

? – *Boletus coloratipes* (Singer) J. Blum, Bulletin de la Société Mycologique de France 85 (4): 560 (1970) ("1969") (nom. inval.)

? – *Krombholziella coloratipes* (Singer) Courtecuisse, Clé de determination macroscopique des champignons superieurs des regions du Nord de la France: 119 (1986) (nom. inval.)

Holotype (*L. brunneogriseolum*): France, Isère, Roybon-St. Etienne de St. Geoir, near Col de Toutes Aures, under *Betula pendula* Roth, 01 Nov 1990, A. Estadès and G. Lannoy, 9011010B (later deposited in the Faculté de Pharmacie de Lille, LIP, see Lannoy & Estadès, 1993, p. 67).

Macroscopic description (**Figs. 2-5**)

Basidiomes medium to less frequently medium-large.

Pileus (5.9) 6.1-10.7 (11.1) cm broad, at first hemispherical then persistenly convex-pulvinate, regularly shaped over the entire life-cycle, fleshy, firm only at the beginning, soon softer and flabby in old basidiomes; margin steady, initially slightly involute then curved downwards and remaining so in aged specimens, extending beyond the tubes up to 1 mm; surface matt, dry but sticky to slightly greasy and polish with moist weather, even to hardly rugulose, very finely pruinose-tomentose in the early stage of development but later smooth and glabrous, not cracked; cuticle with various shades of gray brown, ranging from uniformly brownish, brown grayish to chestnut brown; unchangeable on handling, touching or when injured; subcuticular layer whitish.

Tubes at first extremely thin then increasingly broader and longer than the thickness of the pileus context (up to 2.8 cm long), initially adnate but soon deeply depressed around the stipe apex, whitish to pale beige-cream and further faintly darkening in old fruiting bodies, unchangeable to slowly turning pale flesh-pink when cut.

Pores initially forming a flat surface, later prominently convex, at first tiny to extremely small then gradually wider but always less than 1 mm in diam., simple, persistently roundish, not angular, concolorous with the tubes but with a nearly indistinct grayish halo, very slowly and faintly turning very pale yellowish pink on bruising or when injured and finally becoming light brownish.

Stipe (9.5) 12.0-18.2 (19.5) × 1.2-3.0 (3.2) cm, always tipically longer than the pileus diameter at maturity, slender, central, solid, fibrous, firm, dry, straight or curved especially towards the base to sinuous, cylindrical to sligthly swollen towards the base to subclavate, rounded at the very base, not rooting; surface entirely ornamented by extremely densely arranged, confluent and adpressed, concolorous small asperulations giving the appearance of a woolly velvet throughout, increasingly more pronounced and evident downwards, sometimes also fibrillose at apex, evelate; evenly beige to very pale ochraceous but whitish and with innate blue to aquamarine spots or shades at the base or in the cortex near the base, unchangeable to imperceptibly darkening when pressed; basal mycelium whitish.

Context firm and tough when young, later soft textured and eventually flabby in the pileus (up to 2.1 cm thick in the central zone, up to 1.3 mm thick halfway to margin and gradually becoming thinner towards the edge), fibrous in the stipe, whitish throughout with blue to aquamarine spots or shades at the stipe base or towards the peripheral zones of the lower half of the stipe, tending to become more evident after cutting; unchangeable when exposed to air; whitish to cream colored where eaten by slugs; subhymenophoral layer whitish; exsiccate brownish.

Odor indistinct.

Taste mild.

Spore print not obtained.

Macrochemical spot-test reactions 30% NH_4OH: rusty brown on pores, no reaction elsewhere; 30% KOH: staining wine red on pileus, hymenophore and stipe surface, very pale flesh-pink on context. $FeSO_4$: pale aquamarine everywhere, more intense on the hymenophore

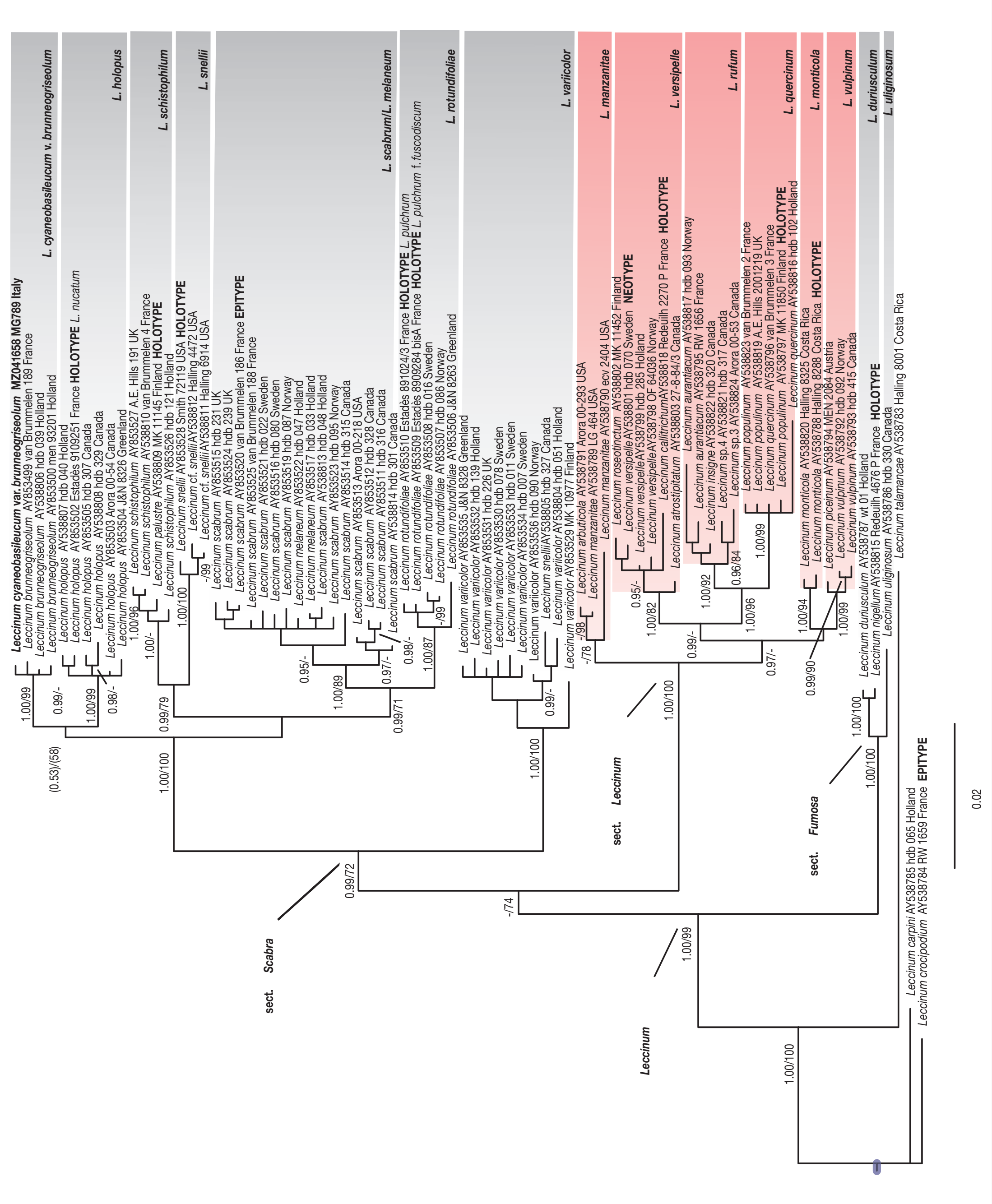

Fig. 1. Phylogeny of the genus *Leccinum* based on a bayesian and maximum likelihood inference analyses of the single nuclear gene region gapdh. Bayesian posterior probability (bpp) values ≥ 0.95 And maximum likelihood bootstrap (mlb) values ≥ 70% are shown above or below the branches. Lower values are exceptionally indicated inside parentheses. The newly sequenced collection is in bold.

Microscopic description (**Plate 1**)

Basidiospores [102/9/3] (15.7) 18.8 ± 1.2 (24.0) × (5.0) 5.8 ± 0.3 (6.9) µm, Q = (2.76) 2.82-3.84 (4.36), Qm = 3.23 ± 0.22, V = 336 ± 49 µm^3 (a single anomalous spore measured 30.0 × 9.0 µm!), fairly variable in dimension, inequilateral, elongated subfusiform to fusiform in side view, elongated fusiform in face view, smooth, apex rounded to slightly pointed, with a short apiculus and without or with a very shallow suprahilar depression, devoid of or with a very slightly pronounced adaxial swelling, moderately thick-walled (0.5-0.8 µm), honey yellow colored in water and 5% KOH, having one, two or three large oil droplets when mature, less frequently pluri-guttulate, inamyloid to very barely dextrinoid, cyanophilic and with an orthochromatic reaction.

Basidia (25) 27-42 (47) × 9-17 µm (n = 23), cylindrical-clavate to clavate, moderately thick-walled (0.5-0.7 µm), predominantly 4-spored but also 1- or 2-spored, usually bearing relatively short sterigmata (2-5 µm), hyaline to pale yellowish and containing straw-yellow oil guttules in water and 5% KOH, bright yellow (inamyloid) in Melzer's, without basal clamps; basidioles subcylindrical to faintly clavate, similar in size to basidia.

Cheilocystidia (28) 32-55 (57) × 4-8 µm (n = 22), very common, moderately slender, projecting straight to sometimes flexuous, irregularly cylindrical or cylindrical-fusiform to fusiform or less frequently ventricose-fusiform and then usually showing a narrow and long neck, with rounded to subacute tip, smooth, moderately thick-walled (0.5-0.8 µm), hyaline to pale yellowish in water and 5% KOH, bright yellow (inamyloid) in Melzer's, without epiparietal encrustations.

Pleurocystidia (51) 53-80 (85) × 7-14 µm (n = 14), unfrequent, color and chemical reactions similar to but more frequently ventricose-fusiform and usually longer and broader than cheilocystidia.

Pseudocystidia not recorded.

Pileipellis a trichoderm to ixotrichoderm consisting of moderately to strongly interwoven, not to rarely branched, somewhat elongated cylindrical hyphae intermixed with abundant, short cylindrical or sausage-like, easily disarticulating hyphae (cylindrocysts) up to 82 × 19 µm, more or less erect in the outermost layer but tending to be repent with age and thus turning into a collapsed cutis embedded in gelatinous matter; terminal elements 25-100 × 5-14 µm, short to relatively long and slender, cylindrical to cystidioid and progressively tapering towards the apex which is rounded-obtuse to pointed, moderately thick-walled (up to 1 µm), nearly colorless or pale yellowish to more often with a golden brownish plasmatic pigment in water and 5% KOH, inamyloid in Melzer's, smooth to occasionally ornamented by a scattered and very subtle granular epiparietal encrustation; subterminal elements similar in shape, size and color to terminal elements.

Stipitipellis a layer of slender, parallel to loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae, 3-10 µm wide, hyaline to pale yellowish in water and 5% KOH; the stipe apex with scattered protruding tufts of caulohymenial elements consisting of sterile caulobasidioles, sparse, predominantly 4-, but also 3-, 2- and 1-spored, subcylindrical to clavate fertile **caulobasidia**, (25) 28-41 (45) × 9-14 µm, sterigmata 3-5 µm long (n = 10), and extremely common projecting **caulocystidia** similar in size, shape and color to hymenial cystidia but also sublageniform or mucronate with blunt apex, sometimes multi-septate, (30) 32-75 (79) × 6-17 µm (n = 14), having a wall up to 1 µm thick.

Lateral stipe stratum under the caulohymenial tufts present and well differentiated from the stipe trama, of the "leccinoid type", (100) 200-300 (400) µm thick layer consisting of inclined, divergent and more or less anticlinally arranged, running outwards from the external surface, predominantly parallel to subparallel, infrequently branched hyphae remaining densely packed and not embedded in a gelatinous substance; the lateral stipe stratum appears to be present only in correspondence to the scabrosities of the stipe surface, absent elsewhere.

Stipe trama composed of confusedly and densely arranged, strongly interwoven, filamentous, smooth, inamyloid hyphae, 2-12 (15) µm broad.

Hymenophoral trama bilateral divergent of the "*Boletus*-type", with slightly to strongly divergent, recurved-arcuate and loosely arranged, sometimes branched, distantly septate and generally not restricted at septa, gelatinous hyphae (lateral strata hyphae in transversal section not touching each other, (2) 3-9 (10) µm apart, 2-10 µm broad), hyaline to very pale yellowish in water and 5% KOH, inamyloid in Melzer's; lateral strata (5) 10-30 (40) µm thick, mediostratum (10) 15-20 (30) µm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 2-7 µm broad, more frequently septate; in Congo Red the mediostratum is darker than the lateral strata.

Oleipherous hyphae very common, golden yellow to brownish in 5% KOH.

Clamp connections absent.

Ontogenetic development gymnocarpic.

Ecology and **phenology** solitary to gregarious, preferably on acidic, dry to humid, sandy, loamy or peaty soil, in water-logged areas or open bogs under *Betula* spp., often in mixed woodlands with other broadleaved trees and conifers, sometimes with *Sphagnum*. Seemingly localized but not uncommon and even frequent in some areas, late spring to autumn (May to November).

Edibility edible but not particularly tasty, the fibrous stipe should be discarded as in all other *Leccinum* species.

Known distribution Likely spanning all over Europe up to boreal, subalpine or subarctic zones but predominantly occurring in temperate regions of central and southern Europe. To date reported from France, England, Scotland, Ireland, Denmark, Norway, Sweden, Finland, Holland, Belgium, Germany, Switzerland, Austria, Czech Republic, Slovakia, Hungary, Russia, Spain, Italy and Serbia. Apparently exotic with introduced birches along the Pacific Northwest (California, USA) and possibly elsewhere in the USA.

Examined material Italy, Latium, Manziana (RM), Cerreta di Manziana, loc. Solfatara, 42°06'55''N, 12°07'21''E, 348 m a.s.l., several specimens in all developmental stages, on loamy and strongly acidic soil, on litter among grass in an opened mixed woodland under *Betula pendula* with the presence of *Quercus cerris* L., *Q. robur* L., *Pinus pinaster* Aiton and *P. pinea* L. in the proximity of a large sulphureous bog, *leg*. M. Gelardi and L. Fabrizi, *det*. M. Gelardi, 01 Nov. 2019, MG789; same loc., 07 Nov. 2019, *leg.* and *det*. M. Gelardi, MG793; same loc., 15 Nov 2019, *leg.* and *det.* M. Gelardi and F. Costanzo, MG797 (dupl. GS10956).

Other species found in the same habitat (not necessarily in the same period of growth): *Amanita fulva* Fr., *Amanita muscaria* (L.) Lam., *Amanita junquillea* Quel., *Amanita rubescens* (Pers.) Gray, *Amanita pantherina* (DC.) Krombh., *Astraeus hygrometricus* (Pers.) Morgan, *Boletus edulis* Bull., *Callistosporium xanthophyllum* (Malençon & Bertault) Bon, *Cordyceps militaris* (L.) Link, *Cortinarius anomalus* Fr., *Gymnopus erythropus* (Pers.) Antonín, Halling & Noordel., *Hygrocybe miniata* (Fr.) P. Kumm., *Imleria badia* (Fr.) Vizzini, *Laccaria amethistina* (Huds.) Cooke, *Laccaria purpureobadia* D.A. Reid, *Lactarius chrysorreus* Fr., *Lactarius helvus* (Fr.) Fr., *Lactarius necator* (Bull.) Pers., *Lactarius quietus* (Fr.) Fr., *Lactarius rufus* (Scop.) Fr., *Lactarius serifluus* (DC.) Fr., *Paxillus involutus* f. *eburneus* Gelardi, Segneri & Vizzini, *Pisolithus* sp., *Rhodocollybia butyracea* (Bull.) Lennox, *Rickenella fibula* (Bull.) Raithelh., *Russula atropurpurea* (Krombh.) Britzelm., *Russula fragilis* Fr., *Russula ochroleuca* Fr., *Scleroderma cepa* Pers., *Scleroderma citrinum* Pers., *Scleroderma verrucosum* (Bull.) Pers., *Suillus bovinus* (L.) Roussel, *Suillus luteus* (L.) Roussel, etc.

Fig. 2. *L. cyaneobasileucum* var. *brunneogriseolum* in habitat (MG793). Photo by Matteo Gelardi

Fig. 3. *L. cyaneobasileucum* var. *brunneogriseolum* in habitat (MG789). Photo by Matteo Gelardi

Fig. 4. *L. cyaneobasileucum* var. *brunneogriseolum* in habitat (MG797). Photo by Matteo Gelardi

Notes

Leccinum cyaneobasileucum Lannoy & Estadès was firstly introduced from south-eastern France (Isère) in the early 90s by the French boletologists A. Estadès and G. Lannoy (Lannoy & Estadès, 1991a), much resembling in its general appearance and very pale tints the well-established, whitish-colored allied species *Leccinum holopus* (Rostk.) Watling. A few months later the same authors described the brown-capped *Leccinum brunneogriseolum* Lannoy & Estadès as a separate species (Lannoy & Estadès, 1991b). In the same issue they also defined *L. brunneogriseolum* var. *pubescentium* Lannoy & Estadès (Lannoy & Estadès, 1991b) and an additional infraspecific taxon was subsequently recognized as *L. brunneogriseolum* f. *chlorinum* Lannoy & Estadès (Lannoy &

Fig. 5. Close-up on the stipe ornamentation of *L. cyaneobasileucum* var. *brunneogriseolum* (MG793) (left) and *L. scabrum* (MG647) (right).
Photo by Matteo Gelardi

Estadès, 1993). Both species were assigned to sect. *Scabra*, subsect. *Pseudoscabra* A.H. Smith, Thiers & Watling emend. Lannoy & Estadès due to the blue-green stains in the stipe and the presence of cylindrocysts in the pileipellis (Lannoy & Estadès, 1994). Nearly fifteen years later, however, Den Bakker & Noordeloos (2005) sequenced samples of both *L. cyaneobasileucum* and *L. brunneogriseolum* and surprisingly they turned out to be conspecific and to merely represent two morpho-chromatic aspects of a single species, being *L. cyaneobasileucum* only an albinotic or light-colored variant of *L. brunneogriseolum* (Den Bakker & Noordeloos, 2005; Den Bakker *et al*., 2007). Indeed, a few years before Lannoy and Estadès hypothesized that *L. cyaneobasileucum* was a "*possible form blanche de brunneogriseolum*" (Lannoy & Estadès, 2001) and later on a "*possible variété blanche de L. brunneogriseolum mais pas d'intermédiaires connus avec ce dernier*" (Estadès & Lannoy, 2004). Unfortunately, being *L. cyaneobasileucum* chronologically earlier this is the correct binomial to be applied at the specific level because, even though *L. cyaneobasileucum* and *L. brunneogriseolum* were described by the same authors (Lannoy and Estadès), in the same year (1991) and in the same journal (Doc. Mycol. 21), they were published in issues 81 (March, but effectively published on May) and 82 (July), respectively (Lannoy & Estadès, 1991a, b). This is therefore an unusual case in which an albinotic form holds nomenclatural priority and takes precedence over its pigmented counterpart. Estadès & Bidaud (2007) introduced the invalid combination *L. brunneogriseolum* var. *cyaneobasileucum* (Lannoy et Estadès) Lannoy et Estadès which was rectified one year later as *L. cyaneobasileucum* var. *brunneogriseolum* (Lannoy et Estadès) Lannoy et Estadès (Lannoy & Estadès, 2008). In recent times Klofac & Krisai-Greilhuber (2016) made two novel combinations, *L. cyaneobasileucum* var. *brunneogriseolum* f. *pubescentium* (Lannoy & Estadès) Klofac and *L. cyaneobasileucum* var. *brunneogriseolum* f. *chlorinum* (Lannoy & Estadès)

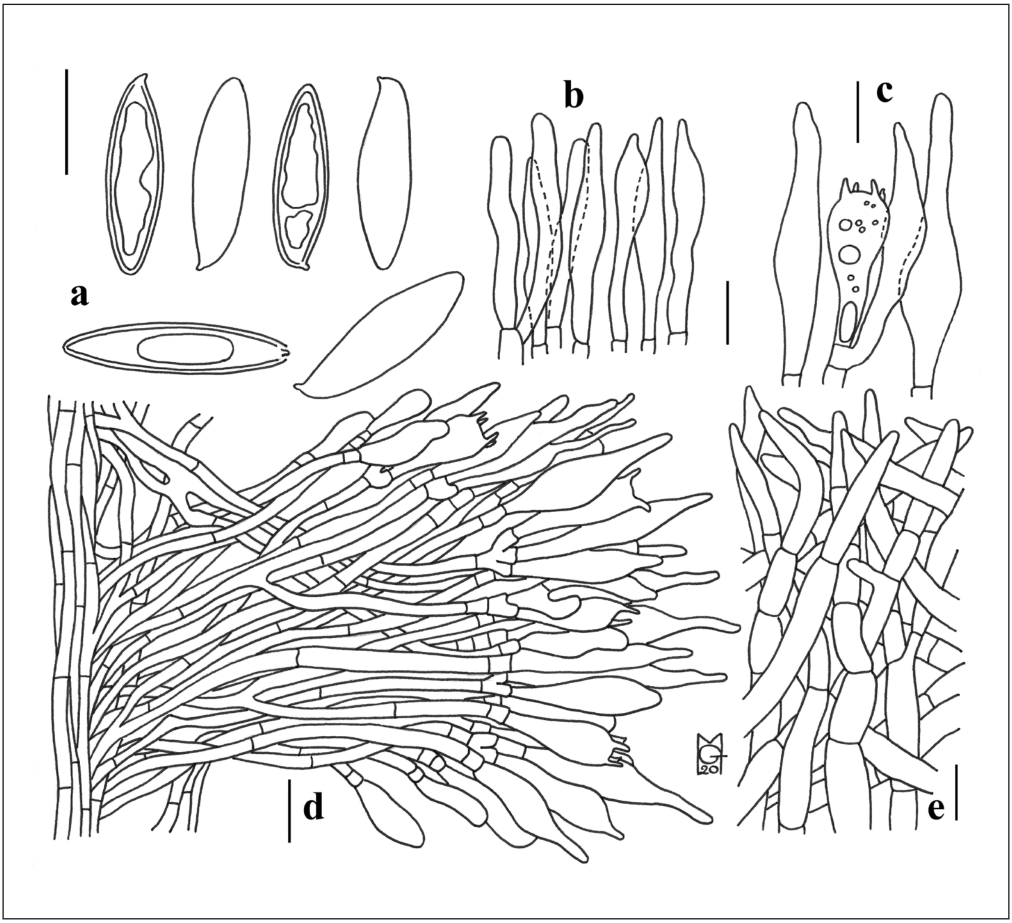

Plate 1. *Leccinum cyaneobasileucum* var. *brunneogriseolum*. Microscopic characters. **a**. Spores; **b**. Cheilocystidia; **c**. Pleurocystidia and basidium; **d**. Stipitipellis; **e**. Pileipellis. Scale bars: **a** = 10 μm; **b-d** = 15 μm; **e** = 20 μm. Drawings by Matteo Gelardi

Klofac, whereas Blanco-Dios (2018) proposed the new combination *Leccinum cyaneobasileucum* var. *pubescentium* (Lannoy & Estadès) Blanco-Dios.

The pigmented phenotype of this species (var. *brunneogriseolum*) produces medium to medium-large, slender basidiomes characterized by a finely pruinose-tomentose, dull brownish gray, brownish to dark brown pileus surface, stipe ornamented by densely arranged, fine squamules resulting in a velvety to nearly woolly appearance, at first pale colored then slightly darkening but never becoming blackish, unchangeable to very faintly pale pinkish context on exposure, discrete and variably extended bluish to greenish stains or blotches at or near the stipe base both internally and externally, cream yellowish colored tissues where eaten by slugs, large basidiospores (on average 18.8 × 5.8 μm, Qm ≥ 3.2), abundant, easily detachable cylindrocysts in the pileipellis and obligate ECM association with *Betula* spp. on acidic soil in temperate, boreal, subalpine to subarctic regions (Lannoy & Estadès, 1991b, 1995; Estadès & Lannoy, 2004; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Calzada Domínguez, 2007; Den Bakker *et al.*, 2007; Cervini, 2009; Šutara *et al.*, 2009; Mikšík, 2017; Noordeloos *et al.*, 2018; Klofac & Krisai-

Greilhuber, 2020). *Leccinum cyaneobasileucum* var. *brunneogriseolum* f. *pubescentium* differs from the typical variety by a more brownish pileus surface without grayish tones, a more robust stipe, some other minor chromatic discrepancies and for being associated with *Betula pubescens* Ehrh. with the presence of *Salix* spp. in peaty-moss places often with *Sphagnum* (Lannoy & Estadès, 1991b, 1995; Estadès & Lannoy, 2004), whereas *L. cyaneobasileucum* var. *brunneogriseolum* f. *chlorinum* exhibits paler tints and olivaceous green hues at pileus margin and over the stipe surface with age (Lannoy & Estadès, 1993, 1995; Estadès & Lannoy, 2004; Muñoz, 2005). Both infraspecific taxa can be simply considered mere morphological and/or ecological or even climatic variants of *L. cyaneobasileucum* var. *brunneogriseolum* of no taxonomic value (Den Bakker & Noordeloos, 2005).

None of the specimens examined herein exhibited any discoloration of the context when cut but in the most relevant literature a pinkish to pale pinkish red staining on exposure is also reported as sometimes occurring, although restricted to the context of the pileus and stipe apex only (Lannoy & Estadès, 1991b, 1995; Estadès & Lannoy, 2004; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Klofac, 2007; Noordeloos *et al*., 2018).

All samples were collected on loamy and strongly acidic soil in a small thicket known as Solfatara in the Macchia Grande di Manziana, a quite peculiar place dominated by *Betula pendula* with the presence of *Pinus pinaster* and *P. pinea* adjacent to a sulphureous bog at the edge of a huge *Quercus cerris* woodland (locally called "Cerreta di Manziana"). This habitat is a restricted ecological niche of extraordinary mycofloristic significance since it represents a natural post-glaciation vegetation relict where a residual but progressively declining population of *Betula pendula* still survive. Such a location is approximately five km away from the Caldara di Manziana, a similar birch-dominated environment where another unique member of the *Boletales* has recently been revealed (Gelardi *et al*., 2011). Curiously, *L. cyaneobasileucum* var. *brunneogriseolum* has never been observed in the Caldara di Manziana, being replaced by the more common *L. scabrum* (Bull.) Gray. This species, by converse, does not appear to be present in the Solfatara.

In the present study we obtained ITS sequences from collections MG789 (MZ041710) and MG797 (MZ041711). However, molecular phylogenetic analyses show that the universal barcoding gene ITS is identical for *L. cyaneobasileucum* and *L. holopus* (data not shown). Such a perfect overlapping of the sequence pair-base can be explained with their ability to hybridize since "*probably recent hybridization between L. cyaneobasileucum and L. holopus and subsequent introgression has resulted in the introduction of a L. holopus ITS in the L. cyaneobasileucum genome*" (Den Bakker & Noordeloos, 2005, p. 523), although this hypothesis would require further testing to be confirmed (Den Bakker *et al.* 2007). Conversely, the *gapdh* sequences of *L. holopus* and *L. cyaneobasileucum* are different and due to a better resolution *gapdh* allows a separation of these two species (Den Bakker *et al*., 2004b, 2007). A *gapdh* sequence was generated from MG789 (MZ041658) and it clearly nested within the clade of *L. cyaneobasileucum* s.l. (**Fig. 1**). Repeated attempts were also made to produce an additional *gapdh* sequence from MG797 but they resulted unsuccessful.

According to Lannoy & Estadès (1991b) and Muñoz (2005), the invalidly published *Krombholzia scabra* var. *coloratipes* Singer (and subsequent combinations) should be considered an earlier heterotypic synonyms of *L. cyaneobasileucum* var. *brunneogriseolum*, however, this taxon has been variously interpreted by different authors and further research would be required to ascertain its conspecificity.

The current known distribution of *L. cyaneobasileucum* s. l. in Europe appears to be somewhat fragmentary, perhaps partly due to misidentification (having been in most cases historically recorded as *L. scabrum*) and/or understudied suitable habitats, nonetheless it has been reported from several countries, including France (Lannoy & Estadès, 1991a, b, 1993, 1994, 1995, also as *L. aboroseolum* (J. Blum) Lannoy & Estadès; Berger, 1992; Estadès & Lannoy, 2004; Courtecuisse & Duhem, 2007; Eyssartier & Roux, 2011), England (Kibby, 2000, 2006, 2011; Watling & Hills, 2005), Scotland (Watling & Hills, 2005), Ireland (First Nature), Denmark (Knudsen & Taylor, 2008), Norway (Discover Life), Finland (Discover Life), Holland (Noordeloos, 1997; Den Bakker, 2000; Noordeloos *et al*., 2018), Belgium (Van De Kerckhove, 2016), Sweden (Knudsen & Taylor, 2008), Germany (Dörfelt H. & Görner H., 1989, as *L. scabrum*; Dähncke, 1993, as *L. onychinum* Watling; Lannoy & Estadès, 1995; Gminder, 1999; Schreiner, 1999; Krieglsteiner, 2000), Switzerland (Cervini, 2009; WLS 2021), Austria (Austrian Mycological Society, 2003-2016), Czech Republic (Šutara *et al*., 2009; Hagara, 2014; Mikšík, 2017), Slovakia (Mikšík, in litt.), Hungary (Siller *et al*., 2013), Russia (Ivanov, 2014) Spain (Muñoz, 1995, 1996, 2000, 2005; Calzada Domínguez, 2007) and Serbia (Discover Life). Due to the relatively few British records and the difficulties in estimating satisfactorily the effective population, *L. cyaneobasileucum* has provisionally been treated as DD (Data Deficient) in the Red List of *Boletaceae* for Great Britain (Ainsworth *et al*., 2013), with the prospect to be assessed as LC (Least Concern), as already proposed by Evans *et al*. (2006). It has also been mentioned as an introduced species from temperate northern California under planted birches on urban lawns (Siegel & Schwarz, 2016) although these records await molecular confirmation. Additional location in the USA are retrievable on Mycoportal. In Italy *L. cyaneobasileucum* s. l. was previously found in alpine and subalpine areas (Piedmont, Liguria, Lombardia, Trentino) (Cervini, 2009; AA.VV., 2015; A.M.I.N.T., 2016). To the best of our knowledge the present communication attests the first molecularly corroborated record of *Leccinum cyaneobasileucum* var. *brunneogriseolum* for central Italy (Latium). Almost certainly this species is commoner and more widespread in Europe than previously assumed.

There exists a strict morphological and ecological correlation between *L. cyaneobasileucum* var. *brunneogriseolum* and the well-known but chromatically fairly variable *L. scabrum* [= *L. roseofractum* Watling, *L. subcinnamomeum* Pilát & Dermek, *L. rigidipes* P.D. Orton, *L. pulchrum* Lannoy & Estadès p.p., etc.], this latter differing by the generally larger size (pileus up to 20 cm diam.), distantly arranged, fine and granulose, grayish brown to blackish scabrosities on stipe surface becoming coarser downwards, stipe tissues always devoid of bluish green spots, absence of cylindrocysts in the pileipellis (although cylindrocysts can occasionally be present in *L. scabrum*!), mostly clavate and usually longer (up to 92 µm) caulocystidia and the occurrence in drier habitats (Lannoy & Estadès, 1995; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Galli, 2013; Van De Kerckhove, 2016). *Leccinum scabrum* var. *melaneum* (Smotl.) Pilát & Dermek, which according to Den Bakker & Noordeloos (2005) might be an autonomous species, differs from *L. cyaneobasileucum* var. *brunneogriseolum* by the very dark brown pileus, the broadly clavate, grayish to brownish stipe with dense and coarse blackish squamules, the stipe base devoid of bluish green discolorations and the pileipellis without cylindrocysts (Lannoy & Estadès, 1995; Den Bakker & Noordeloos, 2005).

Leccinum variicolor Watling s.l. [= *L. oxydabile* (Singer) Singer s. auct. p.p.] is easily distinguished on account of the non-pruinose, mottled dark brown, sepia brown to blackish brown pileus surface often with scattered whitish, beige to pale yellowish ochraceous spots, different stipe ornamentation consisting of isolated and coarser, dark brown to blackish scabrosities, generally stronger reddening of the context upon exposure, context in the stipe base becoming yellowish upon drying, shorter basidiospores [(12) 13.5-18.5 (20.5) × (4.5) 5.0-6.5 (7.0) µm, on average 16.5 × 5.5 µm, Qm ≤ 2.8] and larger caulocystidia (35-125 × 7.5-30 µm) (Watling, 1969; Engel *et al*., 1983; Lannoy & Estadès, 1991a; Lannoy & Estadès, 1995; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Galli, 2013; Van De Kerckhove, 2016).

Another very similar European species *L. schistophilum* M. Bon (= *L. palustre* M. Korhonen) exhibits a paler, cream ochraceous, ochraceous gray to light ochraceous brown pileus, coarser squamules on stipe surface becoming blackish with age, shorter and slightly broader basidiospores [(13) 13.5-17 (22) × (5) 5.5-7.5 (8) µm, on average 16.5 × 6.5 µm, Qm < 3.0], pileipellis hyphae usually with a plaque-like incrusted pigment, mainly utriform, broader caulocystidia (24) 32-62 (80) × (10) 13-28 µm, absence of cylindrocysts in the pileipellis and the occurrence on basic (calcareous) soil in wet, hygrophilous places (Bon & Van Haluwyn, 1981; Korhonen, 1995, as *L. palustre*; Lannoy & Estadès, 1995; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Van De Kerckhove, 2016).

Leccinum rotundifoliae (Singer) A.H. Smith, Thiers & Watling (= *L. scabrum* subsp. *tundrae* Kallio, *L. pulchrum* Lannoy & Estadès, holotype material!, *L. pulchrum* f. *fuscodiscum* Lannoy & Estadès) is promptly separated from *L. cyaneobasileucum* var. *brunneogriseolum* based on the smaller size and stouter basidiomes (pileus up to 10.5 cm diam., stipe up to 10 × 2.5 cm), generally paler, light yellowish brown to ochraceous brown and frequently finely to coarsely cracking pileal surface, stipe base without any blue-green staining, shorter and slightly broader basidiospores [(11.5) 13.5-18 (20) × (4.5) 5.5-7 (7.5) µm, on average 16.9 × 6.4 µm, Qm ≤ 3.0], pileipellis devoid of cylindrocysts and the distribution restricted to cold climates in boreal to (sub)arctic environments of the tundra in the circumpolar region or at lower latitudes in alpine meadows above the timberline, in association with dwarf birches (*Betula nana* L., *B. rotundifoliae* Spach and *B. glandulosa* Michx.) but also with *B. pubescens*. Its occurrence has been confirmed from northern European countries (Greenland, Iceland, Denmark, Norway, Sweden, Finland), Russia but also from the Alps (France) at very high elevations (Lannoy & Estadès, 1995; Den Bakker & Noordeloos, 2005; Muñoz, 2005; Den Bakker *et al*., 2007; Knudsen & Taylor, 2008).

Based on phylogenetic evidence *Leccinum,* sect. *Scabra,* subsect. *Fumosa,* is elevated to the rank of section:

Leccinum sect. ***Fumosa*** (A.H. Smith, Thiers & Watling) Gelardi **comb.** & **stat. nov.**
Mycobank MB 839487

Basionym: *Leccinum* sect. *Scabra,* subsect. *Fumosa* A.H. Smith, Thiers & Watling, Mich. Bot. 6 (3A): 138. 1967 emend. Lannoy et Estadès Doc. Mycol. 24 (94): 11. 1994 (MB538815).

The separation of sect. *Fumosa* from sect. *Leccinum* and sect. *Scabra* was already inferred in previous works (Den Bakker *et al*., 2004a, b, 2007; Den Bakker & Noordeloos, 2005) based on both *gapdh* and the universal barcoding marker ITS, but Den Bakker and colleagues considered sect. *Fumosa* as a subsection of *Leccinum* sect. *Leccinum* and this latter as sister of *Leccinum* sect. *Luteoscabra* Singer (they did not accept the genus *Leccinellum*). However, by recognizing *Leccinellum* as a genus standing on its own all subclades within the *Leccinum* clade (Den Bakker & Noordeloos, 2005; Den Bakker *et al*., 2007) must be treated as sections.

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