The Genus Leccinum (Boletaceae, Boletales) from China Based on Morphological and Molecular Data

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Abstract: Leccinum is one of the most important groups of boletes. Most species in this genus are ectomycorrhizal symbionts of various plants, and some of them are well-known edible mushrooms, making it an exceptionally important group ecologically and economically. The scientific problems related to this genus include that the identification of species in this genus from China need to be verified, especially those referring to European or North American species, and knowledge of the phylogeny and diversity of the species from China is limited. In this study, we conducted multi-locus (nlrLSU, tef1-a, rpb2) and single-locus (ITS) phylogenetic investigations and morphological observations of Leccinum from China, Europe and North America. Nine Leccinum species from China, including three new species, namely L. album, L. parascabrum and L. pseudoborneense, were revealed and described. Leccinum album is morphologically characterized by the white basidioma, the white hymenophore staining indistinct greenish blue when injured, and the white context not changing color in pileus but staining distinct greenish blue in the base of the stipe when injured. Leccinum parascabrum is characterized by the initially reddish brown to chestnut-brown and then pale brownish to brown pileus, the white to pallid and then light brown hymenophore lacking color change when injured, and the white context lacking color change in pileus but staining greenish blue in the base of the stipe when injured. Leccinum pseudoborneense is characterized by the pale brown to dark brown pileus, the initially white and then brown hymenophore lacking color change when injured, and the white context in pileus and stipe lacking color change in pileus but staining blue in stipe when bruised. Color photos of fresh basidiomata, line drawings of microscopic features and detailed descriptions of the new species are presented.

Keywords: boletes; taxonomy; morphology; phylogeny; new taxa

1. Introduction

The genus Leccinum Gray is a species-rich genus of Boletaceae and is characterized by a whitish or yellow hymenophore, a white to cream context unchanging or staining blue or red when injured, a brown to blackish scabrous to dotted squamules on the surface of the stipe, and comparatively long and smooth basidiospores. Generally, most species of the genus are widely spread in the subarctic, boreal, temperate and Mediterranean regions, with a few secondary expansions to the neotropics [1–12]. Species in Leccinum are both ecologically and economically important. Most species of this genus exhibit mycorrhizal host specificity. Species of Leccinum sect. Sabin Smith & Thiers are associated with plants of Betula, while species of L. sect. Fumosa (A.H. Smith, Thiers & Watling) Gelardi are associated with plants of Populus. In L. sect. Leccinum, species are found exclusively associated with plants of Populus (e.g., L. albostipitatum den Bakker & Noordel. and L. insigne
A.H. Sm., Thiers & Watling), Betula (e.g., *L. atrostipitatum* A.H. Sm., Thiers & Watling), Pinaceae (e.g., *L. vulpinum* Watling and *L. piceinum* Pilát & Dermek) and Ericaceae that form arbutoidmycorrhizas (e.g., *L. manzanitae* Thiers and *L. monticola* Halling & G.M. Muell.). However, there are species in section *Leccinum* that are not host specific, i.e., *L. aurantiacum* (Bull.) Gray. This species is associated with plants of *Betula*, *Populus*, *Quercus*, *Salix* and sometimes with *Tilia* [13,14]. Some species of this genus are well-known edible mushrooms, such as *L. quercinum* (Pilát) E.E. Green & Watling, *L. scabrum* (Bull.) Gray and *L. versipelle* (Fr. & Hók) Snell, which are collected in China during the mushroom season.

The genus *Leccinum* was established by Gray in 1821 [13], based on the type species *L. aurantiacum*. Subsequently, more and more mycologists noticed the morphological distinctness and described many new species of this genus. As currently circumscribed, the genus comprises roughly 150 species [1–3,6–56]. North America is the species diversity center of this genus, and in total 118 species have been recorded from this area [19]. Some of the most important works are the serial works of Smith and Thiers [1,15–17], in which three sections of this genus were proposed (L. sect. *Leccinum* Smith & Thiers, L. sect. *Luteoscabra* Smith & Thiers and L. sect. *Scabra*), with 68 species described from Michigan. Twelve species from Central America were described: one species from Belize, eight species from Costa Rica and three species from Colombia [20–24]. In Europe, Singer divided species of this genus into four sections, including two known sections, L. sect. *Luteoscabra* and L. sect. *Leccinum*, and two newly proposed sections, L. sect. *Roseosabra* and L. sect. *Eximia* [3]. In Singer’s infrageneric classification, L. sect. *Scabra*, established by Smith and Thiers, was merged to L. sect. *Leccinum*. Recent molecular phylogenetic evidence has revealed that species of L. sect. *Luteoscabra*, L. sect. *Roseosabra* and L. sect. *Eximia* belong to divergent clades of Boletaceae and represent many new genera (32,52–54). Thus, the genus *Leccinum* is restricted to the section *Leccinum* (Singer’s infrageneric classification) [3]. den Bakker and Noordelos revised the European *Leccinum* species based on morphology and nrLSU sequences and documented sixteen species [14]. In their subsequent study, they treated the three subclades revealed by den Bakker et. al. in L. section *Leccinum* [33,57] as three subsections (viz. L. subsect. *Leccinum*, L. subsect. *Fumosa* A.H. Sm., Thiers & Watling and L. subsect. *Scabra* Pilát & Dermek) [14]. This infrageneric subdivision was followed in the treatment of the genus in this study. In the Southern Hemisphere, four species have been reported, including one from New Zealand and three from Australia [27–29].

In Asia, six species of *Leccinum* have been reported from Malaysia [6]; ten species from Japan [7–10]; and a total of 31 species have been reported from China based on an extensive literature review [34–36,38–52,56]. Among these Chinese species, twelve species, viz. *L. albellum* (Peck) Singer, *L. chromapes* (Frost) Singer, *L. crocipodium* (Letell.) Watling, *L. eximium* (Peck) Singer, *L. extremiorientale* (Lar. N. Vassiljeva) Singer, *L. griseum* (Quel.) Singer, *L. hortonii* (A.H. Sm. & Thiers) Hongo & Nagas., *L. nigrescens* (Richon & Roze) Singer, *L. rubropunctum* (Peck) Singer, *L. rubrum* M. Zang, *L. rugosiceps* (Peck) Singer and *L. subglabripes* (Peck) Singer have been transferred to other genera [5,11,35,52–55]; eight species, viz. *L. duriusculum* (Schulzer ex Fr.) Singer, *L. intusrubens* (Corner) Høil., *L. oxydabile* (Singer) Singer, *L. quercinum*, *L. rufum* (Schaef.) Kreisel, *L. subleucophaeum* E.A. Dick & Snell, *L. subradicatum* Hongo and *L. vorticolor* Watling were reported without specimen support [39–43,49,51]; and eleven species, viz. *L. ambiguum* A.H. Sm. & Thiers, *L. atrostipitatum* A.H. Sm., Thiers & Watling, *L. aurantiacum*, *L. holopus* (Rostk.) Watling, *L. olivaceopallidum* A.H. Sm., Thiers & Watling, *L. poteri* A.H. Sm., Thiers & Watling, *L. roseofractum* Watling, *L. scabrum*, *L. subgranulosum* A.H. Sm. & Thiers, *L. subleucophaeum* var. *minimum* C.S. Bi and *L. versipelle* were reported with specimen citations [34,38,44–48]. Among these eleven species reported with specimen citations, only *L. subleucophaeum* var. *minimum* was originally described from China, and the remaining species were identified as species originally described from Europe and North America based on general morphological similarities. Indeed, a few species described from Europe and North America do occur in China, especially in northeastern and northwestern China. However,
most species found in China have evolved independently in the southern part of China. Thus, identification of the Chinese Leccinum species needs to be reconfirmed.

In this study, we used both morphological data and molecular sequences from the nuclear ribosomal internal transcribed spacer (ITS), the large subunit of the nuclear ribosomal RNA (nrLSU), the translation elongation factor 1-alpha (tef1-α) and the RNA polymerase II second largest subunit (rpb2), together with ecological data to (1) elucidate species diversity of Leccinum in China; (2) evaluate the phylogenetic relationships of species within Leccinum; (3) make morphological and ecological comparisons between closely related species.

2. Materials and Methods

2.1. Taxon Sampling

Nineteen specimens of the genus Leccinum from China were examined. For each collection, a part of the basidioma was dried with silica gel for DNA extraction. The remaining materials were then air-dried at 45–50 °C using an electric food dehydrator. Specimens studied in this work were deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Genera are abbreviated as follows: L. for Leccinum, Le. for Leccinellum, O. for Octaviania, R. for Rossbeevera, Ru. for Rugiboletus, T. for Turmalinea, Ca. for Castanopsis, Li. for Lithocarpus, P. for Pinus and Q. for Quercus.

2.2. Morphological Observation

The macroscopic descriptions are based on the detailed field notes and photographs of fresh basidiomata. Color codes of the form “4B2” indicate the plate, row, and color block from Kornerup and Wanscher [58]. For microscopic studies, the microscopic features of each part of the basidioma were observed under microscope (Leica DM2000, Leica Microsystems, Wetzlar, Germany), including basidiospores, basidia, cheilocystidia, pleurocystidia and pileipellis, using 5% KOH as a mounting medium to revive the dried materials. Microscopic studies follow Zhou et al. [59]. In the description of Basidiospores, the abbreviation n/m/p means n basidiospores measured from m basidiomata of p collections in 5% KOH solution. The notation of the form (a) b–c (d) stands for the dimensions of the basidiospores; the range b–c contains a minimum of 90% of the measured values, a or d given in parentheses stands for extreme values. Q is used to mean “length/width ratio” of a basidiospore in a side view; Qm means average Q of all basidiospores ± sample standard deviation. Measurements of basidiospores, cystidia, basidia and terminal cells in pileipellis are presented as length × width. All microscopic structures were drawn freehand from rehydrated material under the microscope with 10× eyepiece and 100× objective (the total magnification is 1000×).

2.3. Molecular Procedures

Genomic DNA was extracted from silica gel dried materials or herbarium specimens using the CTAB (Cetyltrimethyl ammonium bromide) method [60]. Polymerase chain reactions (PCRs) were performed to amplify partial sequences of nrLSU, tef1-α, rpb2 and ITS using the extracted DNA. The nrLSU region was amplified with primers LROR/LR5 and LROR/LR3 [61]; tef1-α was amplified with primer pair EF1-983F and EF1-1567R [62]; rpb2 was amplified with primers bRPB2-6F and bRPB2-7.1R [63] and ITS was amplified with primer pair ITS1 and ITS4 [64]. Protocols for the polymerase chain reactions (PCRs) and sequencing followed those in Wu et al. [65] and the references therein.

2.4. Sequence Alignments and Phylogenetic Analyses

The newly generated sequences of each locus were blasted in GenBank, and the most closely related sequences (nucleotide identities >95%) were downloaded for further alignment. Sequences were aligned separately for each of the loci using MAFFT v7.130b with the E-INS-I strategy and manually optimized on BioEdit v7.0.9 [66,67]. Two datasets, the ITS dataset and the multi-locus (nrLSU + tef1-α + rpb2) dataset, were analyzed using RAxML and Bayesian methods, respectively. For the multi-locus dataset, single-gene analyses were conducted to assess incongruence among individual genes using the ML
method (results not shown). Because no well-supported bootstrap value (BS > 70%) [55] conflict was detected among the topologies of the three genes, their sequences were then concatenated together for further multi-locus analyses.

For ML analyses, the multi-locus and ITS datasets were analyzed using RAxML (https://www.phylo.org/, accessed on 26 August 2021) under the model GTRGAMMA [68]. Statistical supports for the phylogenetic analyses were determined using nonparametric bootstrapping with 1000 replicates. For BI analyses, the parameter model was selected by the Akaike information criterion (AIC) as the best-fit likelihood model with Modeltest 3.7 (Free Software Foundation, Boston, MA, USA) [69]. The models employed for each of the four loci were GTR + I + G for ITS, nrLSU and tef1-α, and SYM + I + G for rpb2. Posterior probabilities (PP) were determined twice by running one cold and three heated chains in parallel mode, saving trees every 1000th generation. Other parameters were kept at their default settings. Runs were terminated once the average standard deviation of split frequencies went below 0.01 [70]. Chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/, accessed on 26 August 2021) to confirm sufficiently large ESS values (>200). Subsequently, the sampled trees were summarized after omitting the first 25% of trees as burn-in using the ‘sump’ and ‘sumt’ commands implemented in MrBayes.

3. Results

3.1. Molecular Phylogenetic Analysis

A total of 57 sequences, including fifteen for nrLSU, fifteen for tef1-α, fourteen for rpb2 and thirteen for ITS, were newly generated in this study and aligned with sequences downloaded from GenBank. Sequences retrieved from GenBank and obtained in this study for the multi-locus phylogenetic analyses are listed in Table 1. The multi-locus dataset (Supplementary File S1) contained 122 sequences (49 for nrLSU, 41 for tef1-α, 32 for rpb2), representing 51 samples, and the alignment contained 2195 nucleotide sites, of which 530 were parsimony informative. Borofutus dhakanus Hosen & Zhu L. Yang and Spongiforma thailandica Desjardin, Manfr. Binder, Roekring & Flegel were chosen as the outgroup [71,72]. ML and Bayesian analyses produced very similar estimates of tree topologies, and thus only the tree inferred from ML analysis is displayed (Figure 1). The monophyly of Leccinum was highly supported (BS = 100% and PP = 1) in our analyses. Four main clades were recovered, and three of them correspond to the three known subsections, viz. L. subsect. Leccinum [14]. Three new species, namely L. album, L. parascabrum and L. pseudoborneense, were revealed in our multi-locus phylogenetic analyses. Leccinum parascabrum formed the remaining clade with BS = 100% and PP = 1, while L. pseudoborneense and L. album nested in L. subsect. Scabra of L. sect. Leccinum [14]. Three new species, namely L. album, L. parascabrum and L. pseudoborneense, were revealed in our multi-locus phylogenetic analyses. Leccinum parascabrum formed the remaining clade with BS = 100% and PP = 1, while L. pseudoborneense and L. album nested in L. subsect. Scabra and clustered together with L. flavostipitatum E.A. Dick & Snell, L. subradicatum and L. variicolor with low supported lineage (BS = 54%).

Table 1. Information on specimens used in multi-locus phylogenetic analyses and their GenBank accession numbers. Sequences newly generated in this study are indicated in bold.

| Species         | Voucher | Locality | GenBank Number                  | Reference |
|-----------------|---------|----------|---------------------------------|-----------|
|                 |         |          | ITS | nrLSU | tef1-α | rpb2 |
| Leccinellum corsicum | Buf 4507 | USA      | -   | -     | KF030347 | KF030435 | -   | [55] |
| Le. crocipodium | MICH-KUO-07050707 | USA | - | - | MK601749 | MK721103 | MK766311 | [5] |
| Le. aff. griseum | KPM-NC-0017381 | Japan | - | - | JN378508 | JN378449 | - | [73] |
| Le. lepidum     | K(M)-142974 | Italy | - | - | MK601751 | MK721105 | MK766312 | [5] |
| L. pseudosacrum | CFMR-DPL-11432 | USA | - | - | MK601752 | MK721106 | MK766313 | [5] |
| L. rugosiceps   | CFMR-BOS-866 | USA | - | - | MK601770 | MK721124 | MK766329 | [5] |
| Leccinum album  | KUN-HKAS53417 | China | MZ392872 | MW439287 | MW439267 | MW439259 | This study |
| L. aurantiacum  | L-0342207 | France | - | - | MK601759 | MK721113 | MK766318 | [5] |
| L. cerinum      | MK11800 | Finland | - | - | AF139692 | - | - | [4] |
| L. duriaeum     | KUN-HKAS101160 | Uzbekistan | MZ485402 | MZ275541 | MZ707785 | MZ707779 | This study |
| L. duriaeum     | GL4676 | France | - | - | AF139699 | - | - | [4] |
| Species              | Voucher         | Locality         | ITS nrLSU      | rDNA ITS       | rPB2           | Reference                  |
|---------------------|-----------------|------------------|----------------|----------------|----------------|----------------------------|
| L. flavostipitatum  | MENMB10801       | USA              |               | M6120342       | -              | GenBank                    |
| L. holopus          | MH:KU09150707   | USA              | -             | MK601763       | MK721117       | MK766322 [5]               |
| L. holopus          | 9108320          | France           | -             | AF139700       | -              | -                          |
| L. holopus          | KUN-HKASI11906   | Austria          | -             | MW413906       | MW439266       | MW439258 [5]               |
| L. manzanita         | NY-14011         | USA              | -             | MK601675       | MK721119       | MK766324 [5]               |
| L. melanosporum     | KUN-HKASI57220   | China            | MZ484509      | MZ675542       | MZ707786       | MZ707780 [5]               |
| L. monticola        | NY-0815448       | Costa Rica       | -             | MK601677       | MK721121       | MK766326 [5]               |
| L. monticola        | NY-760388        | Costa Rica       | -             | MK601766       | MK721120       | MK766325 [5]               |
| L. palustre         | MK11107          | Germany          | -             | AF139701       | -              | [5]                        |
| "L. palustre"       | hdb030           | Netherlands      | AF454586      | -              | -              | [57]                       |
| L. parasacrum       | KUN-HKAS99903    | China            | MZ392874      | MW413911       | MW439271       | MW439264 [5]               |
| L. parasacrum       | KUN-HKAS59447    | China            | MZ392875      | MW413912       | MW439272       | MW439265 [5]               |
| L. pseudoborneense  | KUN-HKASI10156   | China            | MZ412902      | MW413908       | MW439268       | MW439261 [5]               |
| L. pseudoborneense  | KUN-HKASI10157   | China            | MZ412903      | MW413909       | MW439269       | MW439262 [5]               |
| L. pseudoborneense  | KUN-HKASI10158   | China            | MZ412904      | MW413910       | MW439270       | MW439263 [5]               |
| L. pseudoborneense  | KUN-HKASI89139   | China            | -             | MZ536631       | MZ543306       | MZ543308 [5]               |
| L. pseudoborneense  | KUN-HKASI92401   | China            | -             | MZ536632       | MZ543307       | MZ543309 [5]               |
| L. quercinum        | KUN-HKAS63502    | China            | -             | KF112724       | KF112250       | KF112724 [65]              |
| L. quercinum        | Lq1              | Germany          | DQ534612      | -              | -              | [74]                       |
| "L. scabrum"        | KUN-HKAS56371    | China            | -             | KT990857       | KT990782       | KT990423 [11]              |
| L. scabrum          | KUN-HKAS57266    | China            | -             | KF112442       | KF112248       | KF112722 [65]              |
| L. scabrum          | KUN-HKASI98029   | China            | MZ485407      | MZ675543       | MZ707787       | -                          |
| L. scabrum          | KPM-NC-0017840   | UK               | -             | JN378515       | JN378455       | -                          |
| L. schistophiliun   | KUN-HKASI98024   | China            | MZ503508      | MZ675544       | MZ707788       | -                          |
| L. schistophiliun   | VDKO1128         | Belgium          | -             | KT824055       | KT824022       | -                          |
| L. subradiatum      | KPM-NC-34518     | Japan            | MT934814      | MT912736       | MT874822       | -                          |
| L. sp.              | KPM-NC-0017830   | Japan            | KC552009      | -              | -              | [77]                       |
| L. varicolor        | Lvar1            | Germany          | KF112443       | KF112249       | KF112723       | -                          |
| L. varicolor        | hdb327           | Canada           | -             | -              | -              | [33]                       |
| L. versipelle       | KUN-HKASI76669   | China            | -             | KF112443       | KF112249       | KF112723 [65]              |
| L. versipelle       | CFMR:DLC2002-122 | USA              | -             | MK601778       | -              | [57]                       |
| L. versipelle       | CFMR:DLC2002-122 | Sweden           | AF454573      | -              | -              | [57]                       |
| L. violaceotinctum  | KUN-HKASI97997   | China            | MZ485404      | MZ675545       | MZ707789       | MZ707781 [5]               |
| L. violaceotinctum  | KUN-HKAS99380    | China            | MZ485401      | MZ675546       | MZ707790       | MZ707782 [5]               |
| L. violaceotinctum  | CFMR:BZ-1676     | Belize           | -             | MK601780       | MK721133       | MK766337 [5]               |
| L. violaceotinctum  | CFMR:BZ-3169     | Belize           | -             | MK721134       | MK766338       | -                          |
| Borofutus dhakanus  | KUN-HKASI73789   | Bengal           | -             | JQ928616       | JQ928576       | JQ928597 [72]              |
| Chondrostereum      | DBG:023599       | USA              | -             | MK601728       | MK721082       | MK766290 [5]               |
| Octavia             | KPM-NC-0017812   | Japan            | -             | JN378486       | JN378428       | -                          |
| japonophytontana    | NY-02494788      | USA              | -             | MK601798       | MK721152       | MK766355 [5]               |
| O. tasmanica        | GDMG4913         | Sweden           | -             | M1337793       | -              | [79]                       |
| Rossbeevera griseobrunnea | KPM-NC-0023895 | Japan            | -             | KP222896       | KP222915       | -                          |
| R. eucanea          | NY-00796145      | USA              | -             | MK601758       | MK721112       | MK766317 [5]               |
| Rugioleus ancinus   | NY-181460        | USA              | -             | MK601757       | MK721111       | MK766316 [5]               |
| Spongiforma         | DED7873          | Thailand         | -             | NG_042464      | KF030436       | MG212648 [71]              |
| Thailandica         | KPM-NC-0017743   | Japan            | -             | KC552050       | -              | -                          |
| T. yuwanensis       | KPM-NC0023377    | Japan            | -             | KJ001098       | KJ001083       | -                          |
| Tyloclumus griseolus| KUN-HKASI52612   | China            | -             | KT990631       | KT990825       | -                          |
Figure 1. Maximum-likelihood phylogenetic tree generated from a three-locus (nrLSU + tef1-α + rpb2) dataset. BS > 50% in ML analysis and PP > 0.95 in Bayesian analysis are indicated as RAxML BS/PP above or below supported branches. Species of this genus from China and type species of this genus (L. aurantiacum) are indicated in bold. Voucher specimens and localities where the specimens were collected are provided behind the species names. AU = Austria, BE = Belgium, CN = China, CR = Costa Rica, FI = Finland, FR = France, GER = Germany, IT = Italy, JP = Japan, TAI = Thailand, UK = United Kingdom, USA = United States of America and UZ = Uzbekistan.

For the ITS dataset, as revealed by den Bakker et al. [57] and our primary analysis, the ITS1 region contains a minisatellite, which is characterized by the repeated presence of CTATTGAAAAG and CTAATAGAAAG core sequences and mutational derivatives. Moreover, some species contain a minisatellite in the ITS2 region, e.g., the newly described species L. album (GenBank Acc. No.: MZ392872 for clone 1 and MZ392873 for clone 2), with a region of 212 bp that consists of tandem repeats (see Supplementary Material for details). Though there is length variation in either the ITS1 or ITS2 spacers, it can also provide some phylogenetic signals. We performed phylogenetic analyses of the ITS dataset. In this dataset (Supplementary File S2), 51 samples were included. The length of the dataset was 1416 bp, of which 377 were parsimony informative. Lecchinellum albellum (Peck) Bresinsky & Manfr. Binder was chosen as outgroup. ML and Bayesian analyses also produced very similar estimates of tree topologies, and only the tree inferred from ML analysis is displayed (Figure 2). The monophyly of Lecchinellum was also well supported (BS = 100% and PP = 1) in our analyses. Three new species viz. L. album, L. parascabrum and L. pseudoborneense) were revealed. Lecchinellum album is closely related to L. pseudoborneense yet without statistical support, while L. parascabrum forms an independent lineage. Species to which L. parascabrum is phylogenetically related remain as yet unknown.
Figure 2. Maximum-likelihood phylogenetic tree generated from ITS dataset. BS > 50% in ML analysis is indicated above or below supported branches. Species of this genus from China and type species of this genus (L. aurantiacum) are indicated in bold. Voucher specimens, localities and GenBank numbers are provided behind the species names. AT = Austria, CAN = Canada, CN = China, CR = Costa Rica, FR = France, GL = Greenland, JP = Japan, NL = The Netherlands, PL = Poland, SW = Sweden, UK = United Kingdom, USA = United States of America and UZ = Uzbekistan.

Our ML and Bayesian analyses of ITS and multi-locus datasets revealed the existence of eight Leccinum species from China, including five known species viz. L. melaneum (Smotl.) Plát & Dermek, L. quercinum, L. scabrum, L. schistophilum and L. versipelle and three new species viz. L. album, L. parascabrum, and L. pseudoborneense. The final alignments of both datasets were deposited in TreeBASE (S27490).

3.2. Taxonomy

Leccinum album X. Meng, Yan C. Li & Zhu L. Yang, sp. nov., (Figures 3g–h and 4). MycoBank: MB 838917.

Diagnosis: This species differs from other species in Leccinum in the combination of the entirely white pileus, the white pileal context not changing color when injured, the white hymenophore staining indistinct greenish blue when hurt, the white stipe coarsely covered with initially white and then darkened verrucose squamules, and the white stipe context always staining greenish blue at the base when injured.

Holotype: CHINA. Hunan Province: Chenzhou, Zhanghua County, Mangshan National Forest Park, E 112°92′, N 24°94′, alt. 850 m, associated with Castanopsis fissa, Cyclobalanopsis glauca, Lithocarpus glabra and Pinus kwangtungensis, 3 September 2007, Y.C. Li1072 (KUN-HKAS53417, GenBank Acc. No.: MZ392872 and MZ392873 for ITS, MW439267 and MW439259 for rpb2).
**Figure 3.** Basidiomata of *Leccinum* species. (a–c) *Leccinum parascabrum* (KUN-HKAS99903, holotype); (d–f) *Leccinum pseudoborneense* ((d) from KUN-HKAS110157; (e,f) from KUN-HKAS110156, holotype); (g,h) *Leccinum album* (KUN-HKAS53417, holotype).

**Etymology:** Latin “*album*” means white, referring to the color of the basidiomata.

Basidiomata small to medium-sized. Pileus 3–5.5 cm in diam., hemispherical when young, subhemispherical to convex or plano-convex when mature, white (1A1) when young, white to cream (2B2–3) when mature; surface covered with concolorous farinose to pubescent squamules; context 5–10 mm thick in the center of pileus, taste mild, white (1A1) to pallid, not changing color when bruised; Hymenophore adnate when young, adnate to slightly depressed around apex of stipe; surface white (1A1), staining indistinct greenish blue (25B5–7) when injured; pores subangular to roundish, 0.3–1.5 mm wide; tubes up to 5 mm long, white to dirty pinkish (13A2), not changing color when bruised. Stipe 8–10 × 0.8–1.2 cm, clavate to subcylindrical, always enlarged downwards; surface white (1A1), densely covered with white (1A1) verrucose squamules, staining light greenish blue at base when injured; context whitish (1A1), staining blue at base when injured; basal mycelium white (1A1), lacking color change when injured.
Basidiospores (40/2/1) 15–19 × 5–7 µm, Q = 2.5–3, $Q_m = 2.75 \pm 0.15$, subfusiform to narrowly ellipsoid in side view with slight suprahilar depression, subcylindrical to fusiform in ventral view, smooth, somewhat slightly thick-walled (up to 0.5 µm thick), hyaline to yellowish in KOH, brownish yellow to olivaceous brown in Melzer’s Reagent. Basidia 23–33 × 10–13 µm, clavate, 4-spored, hyaline to yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Hymenophoral trama boletoid, hyphae subcylindrical, 4–10 µm wide, hyaline to yellowish in KOH, yellowish to yellow in Melzer’s Reagent. Cheilocystidia and pleurocystidia 42–60 × 11–17.5 µm, abundant, subfusiform to fusiform, thin-walled, yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Pileipellis a trichoderm, composed of more or less vertically arranged 5–10 µm wide hyphae, hyaline to yellowish in KOH, yellowish to yellow in Melzer’s Reagent. Pileal trama made up of 6–12 µm wide filamentous hyphae, thin-walled, yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Clamp connections absent in all tissues.

Habitat and distribution: Solitary or scattered in tropical forests dominated by plants of the families Fagaceae (Castanopsis fissa, Cyclobalanopsis glauca and Lithocarpus glabra) and Pinaceae (Pinus kwangtungensis or P. armandii); on acidic, humid and loamy soils;
distribution insufficiently known, rather rare in China and currently found in central and southeastern China (Hunan and Fujian Provinces).

Additional Specimen examined: CHINA. Fujian Province: Jianning County, E 116°84′, N 26°83′, alt. 900 m, associated with *Castanopsis fissa*, *Cyclobalanopsis glauca* and *Pinus armandii*, 16 July 1971, N.L. Huang 716 (KUN-HKAS39522).

Commentary: *Leccinum album* is characterized by the white pileus, the white hymenophore staining indistinct greenish blue when hurt, the white stipe densely covered with initially white and then darkened scabrous squamules, the white context in pileus not changing color when injured, and the white context in stipe unchanging or only staining distinct greenish blue at base when injured. Morphologically, *L. album* is close to *L. holopus*, *L. cyaneobasileucum* Lannoy & Estadès and *Le. albellum* (Peck) Bresinsky & Manfr. Binder in similar pileus colors. However, *L. holopus*, originally described from Europe (Germany), differs from *L. album* in its medium to large basidiomata (pileus 4–10 cm wide), becoming more viscid pileus with age, pure white or dirty white to pale buff or pale pallid pileus always with a glaucous green tinge, long hymenophoral tubes measuring 9–15 mm long, narrow and subcylindrical hymenial cystidia measuring 30–50 × 7.5–12.5 µm, narrow pileipellis hyphae measuring 3.5–5 µm wide, and association with trees of the genus *Betula* (Betulaceae) [80–82]. *Leccinum cyaneobasileucum*, originally described from France, is different from *L. album* in its white or greyish brown to light brown pileus, woolly stipe surface, slender basidiospores with Qm ≥ 3, relatively narrow hymenial cystidia measuring 32–44 × 5.5–7.5 µm, narrow pileipellis hyphae measuring 2–6.5 µm wide, and association with trees of the genus *Betula* [83]. *Leccinellum albellum*, originally described from New York, is characterized by its basidiomata not changing color when bruised and narrow basidiospores measuring 13–20 × 4–6 µm [16,17,30].

Phylogenetically, *L. album* is related to *L. varicolor* and *L. pseudoborneense* in the analyses of the multi-locus and ITS datasets, respectively (Figures 1 and 2). However, *L. varicolor* differs from *L. album* in its white to grey or cream pileal context staining vinaceous to brown when bruised, white stipe context staining pink to coral red in the upper part and green-blue in the lower part when bruised and association with plants of *Betula* [81]. *Leccinum pseudoborneense* is different from *L. album* in its pale brown to dark brown pileus, white context in pileus and stipe staining blue when bruised, narrow basidiospores measuring (11) 12–19 (20) × 4–5 (6) µm, narrow hymenial cystidia measuring 28–40 × 4–10 µm, and distribution in southwestern China.

**Leccinum parascabrum** X. Meng & Yan C. Li & Zhu L. Yang, sp. nov., (Figures 3a–c and 5).

MycoBank: MB 838916.

Diagnosis: This species differs from other species in *Leccinum* by its initially reddish brown to chestnut-brown and then brown to pale brownish or even dirty white pileus, white pileal context lacking color change when injured, white to pallid and then light brown hymenophore lacking color change when injured, and the white stipe context staining greenish blue at the base when injured.

Holotype: CHINA. Hunan Province: Chenzhou, Zhanghua County, Mangshan National Forest Park, E 112°92′, N 24°94′, alt. 1100 m, associated with *Castanopsis fissa*, *Lithocarpus glabra* and *Pinus kwangtudgensis*, 12 September 2016, G. Wu 1784 (KUN-HKAS99903, GenBank Acc. No.: MZ392874 for ITS, MW413911 for nrLSU, MW439271 for *rpb2* and MW439264 for *tef1-a*).

Etymology: Latin “parascabrum” refers to its similarity to *L. scabrum*.

Basidiomata small to medium-sized. Pileus 2.5–12.5 cm in diam., hemispherical when young, subhemispherical to convex or applanate when mature, reddish brown (12E8) to chestnut-brown (8C7–8) when young, brown (6C6) to pale brownish (7D7–8) or even dirty white (6A2) when mature; surface tomentose; context 6–13 mm thick in the center, white (1A1), not changing color when bruised; Hymenophore adnate when young, adnate to slightly depressed around apex of stipe; surface white to pallid (1A1) when young, and becoming light brown (6B4) when mature, not changing color when injured; tubes 6–14 mm long, 0.5–1.5 mm wide, creamy white (1A1), not changing color when bruised.
Stipe 12–14 × 1.1–2.2 cm, clavate, swollen downwards, always staining greenish blue at base when injured; surface white (1A1), covered with initially white (1A1) to light beige (5A4) and then brownish (7D8) squamules; context white (1A1), staining greenish blue (25B6–7) at base when injured; basal mycelium white (1A1).

Figure 5. Microscopic features of Leccinum parascabrum (KUN-HKAS99903, holotype). (a) Basidiospores. (b) Basidia and pleurocystidium. (c) Cheilocystidia. (d) Pleurocystidia. (e) Pileipellis. Bars = 10 µm. Drawings by Y.-C. Li.

Basidiospores (80/2/2) 16–20 (–21) × 5–6 µm, Q = 3.2–3.8, Q_m = 3.43 ± 0.18, subfusiform to fusiform, slightly thick-walled (up to 0.5 µm thick), yellowish to yellowish brown in KOH, yellow to yellow-brown in Melzer’s Reagent. Basidia 24–33 × 8–12 µm, clavate, 4-spored, hyaline to yellowish in KOH, yellowish to yellow in Melzer’s Reagent. Hymenophoral trama boletoid, hyphae cylindrical, 3–7 µm wide, hyaline to yellowish in KOH, yellowish to yellow in Melzer’s Reagent. Cheilo- and pleurocystidia 34–68 × 7.5–16 µm, abundant, subfusiform to fusiform, thin-walled, yellowish to pale yellowish brown in KOH, yellowish brown to brown in Melzer’s Reagent. Pileipellis a trichoderm, composed of 5–9 µm wide filamentous hyphae, yellowish to pale brownish in KOH. Pileal trama made
up of 5–10 µm wide filamentous hyphae, thin-walled, hyaline to yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Clamp connections absent in all tissues.

**Habitat and distribution:** Solitary or scattered in tropical forests dominated by plants of the families Fagaceae (Lithocarpus glabra, Castanopsis fissa and Ca. hystrix) and Pinaceae (Pinus kwangtudgensis or P. yunnanensis); on acidic or slightly alkaline, loamy soils; distribution insufficiently known, rather rare in China, currently known from central and southwestern China (Hunan and Yunnan Provinces).

**Additional Specimen examined:** CHINA. Yunnan Province: on the way from Tengchong County to Longling County, E 98°59′, N 24°81′, alt. 2010 m, associated with Lithocarpus glabra, Castanopsis hystrix and Pinus yunnanensis, 19 July 2009, Y.C. Li 1700 (KUN-HKAS59447, GenBank Acc. No.: MZ392875 for ITS, MW439212 for nrLSU, MW439272 for tefl-α, and MW439265 for rpb2).

**Commentary:** Leccinum parascabrum is characterized by the initially reddish brown to chestnut-brown and later brown to pale brownish or even dirty white pileus, the white pileal context not changing color when injured, the white to pallid and then light brown hymenophore not changing color when injured, the white stipe context with greenish blue color change at the base when injured, and the relatively large basidiospores measuring 16–20 (–21) × 5–6 µm, Q = 3.2–3.8. Leccinum parascabrum generally shares the similar colors of pileus and hymenophore, and the similar slender stems with L. duriusculum, L. griseonigrum A.H. Sm., Thiers & Watling, L. scabrum, L. uliginosum A.H. Sm. & Thiers and Le. pseudoscabrum (Kallenb.) Mikšík. However, L. duriusculum, originally described from Europe, can be distinguished from L. parascabrum by its pale grey-brown to dark greyish or reddish brown pileus, white context staining violaceous pink when bruised but yellow-green to blue-green in the base of stipe, relatively small basidiospores measuring 11.5–15.5 × 4.5–6 µm [84]. Leccinum griseonigrum, originally described from North America, differs from L. parascabrum in its avellaneous to dingy cinnamon-buff pileus, white pileal context staining blue when bruised, relatively small basidiospores measuring 13–16 × 4–5.5 µm, and association with trees of the genus Populus [16]. Leccinum scabrum differs from L. parascabrum in its wrinkled pileus, pale white hymenophore, pinkish discoloration when injured, and never bluish color change at the base of stipe [13,14,81]. Leccinum uliginosum, originally described from North America, is different from L. parascabrum in its dark fuscous to drab-grey pileus, white context in pileus becoming reddish and then fuscous when bruised, relatively small basidiospores measuring 14–18 × 3.5–5 µm, and small and inconspicuous hymenial cystidia [17]. Leccinellum pseudoscabrum differs from L. parascabrum in its initially red to purplish brown and then blackish brown context color change when injured, and the palisadoderm pileipellis composed of subglobose cells [14]. Leccinum parascabrum also shares the similar colors of pileus and hymenophore and the bluish color change at the base of stipe with L. variicolor. However, L. variicolor is different from L. parascabrum in its white to grey or cream pileal context staining vinaceous to brown when bruised, white stipe context staining pink to coral red in the upper part and green-blue in the lower part when bruised, relatively small basidiospores measuring (10) 13.5–17.5 (–20.0) × 5.0–6.5 (7.0) µm with Q = 2.4–3.1, and association with plants of Betula sp. [81]. In our phylogenetic analysis of the multi-locus and ITS datasets (Figures 1 and 2), L. parascabrum formed independent clades within Leccinum. It might represent a distinct section or subsection. However, formal change of the infrageneric division of this clade should await more molecular and morphological data from additional taxa. Species to which it is phylogenetically related remain as yet unknown.

**Leccinum pseudoborneense** X. Meng & Yan C. Li & Zhu L. Yang, sp. nov., (Figures 3d-f and 6).
**Figure 6.** Microscopic features of *Leccinum pseudoborneense* (KUN-HKAS110156, holotype). (a) Basidiospores. (b) Basidia and pleurocystidia. (c) Pleurocystidia. (d) Cheilocystidia. (e) Pileipellis. Bars = 10 µm. Drawings by Y.-C. Li.

**MycoBank:** MB 838915.

**Diagnosis:** This species differs from other species in *Leccinum* in its nearly glabrous and pale brown to dark brown pileus, white context in pileus lacking color change when injured, white context in stipe staining blue when bruised, initially white and then brown hymenophore not changing color when injured, white stipe covered with ochraceous to dark brown squamules, and trichodermal pileipellis composed of 3–6 µm wide interwoven hyphae.

**Holotype:** CHINA. Yunnan Province: Xishuangbanna, Menghai County, Bada Town, E 100°12', N 21°83', alt. 1900 m, associated with *Castanopsis calathiformis*, *Castanopsis indica* and *Lithocarpus truncatus*, 22 June 2020, G.S. Wang 947 (KUN-HKAS110156, GenBank Acc. No.: MZ412902 for ITS, MW413908 for nrLSU, MW439268 for *tef1*-α, and MW439261 for *rpb2*).
Etymology: Latin “pseudo” = false, “borneense” = L. borneense, “pseudoborneense” is proposed because this species is similar to the species L. borneense originally described from Malaysia.

Basidiomata small to medium-sized. Pileus 4–10 cm diam, subhemispherical to convex or plano-convex; surface nearly glabrous, viscid when wet, pale brown (6D6–C5) to dark brown (6F6–E5); context 5–10 mm thick in the center, white (1A1), not changing color when bruised; Hymenophore adnate to depressed around apex of stipe; white (1A1) to pallid when young and becoming brown (6E7) when mature, not changing color when injured. Tubes 4–10 mm long, creamy white (1A1) when young, and becoming brownish yellow (5C7–8) when mature, not changing color when bruised; pores fine, no more than 1 mm wide. Stipe 2.1–2.9 cm, clavate, always swollen downwards; surface white (1A1), covered with ochraceous (2B3–5) to dark brown (6E7) squamules, staining asymmetric blue (23E7) when injured; context white (1A1), staining blue (23E7) when injured; basal mycelium white (1A1).

Basidiocarpus (100/5/5) (11–) 12–19 (–20) × 4–5 (–6) µm, Q = (2.75–) 3–3.58 (–3.6), Qm = 3.31 ± 0.16, subfusiform to ellipsoid, slightly thick-walled (up to 0.5 µm thick), yellowish brown to olive brown in KOH, yellow-brown to dark olive-brown in Melzer’s Reagent. Basidia 18–30 × 8–9 µm, clavate, 4-spored, hyaline to yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Hymenophoral trama boletoid, hyphae cylindrical, 3–6 µm wide, hyaline to yellowish in KOH, yellowish to brownish yellow in Melzer’s Reagent. Cheilo- and pleurocystidia 28–40 × 4–10 µm, abundant, subfusiform to fusiform, thin-walled, yellowish to brownish yellow in KOH, brownish to yellow-brown in Melzer’s Reagent. Pililepellis a trichoderma, composed of more or less vertically arranged 5–12 µm wide filamentous hyphae, yellowish brown to brownish in KOH, brown to dark brown in Melzer’s Reagent. Pilileal trama made up of 6–12 µm wide filamentous hyphae, thin-walled, hyaline to yellow in KOH, yellowish to yellow in Melzer’s Reagent. Clamp connections absent in all tissues.

Habitat and Distribution: Scattered in tropical forests dominated by plants of the families Fagaceae (Castanopsis calathiformis, Ca. orthacantha, Ca. indica, Lithocarpus truncatus, Li. mairei and Quercus griffithii); on acidic, loamy or mossy, humid soils; moderately common in southwestern China (Yunnan Province).

Additional specimens examined: CHINA. Yunnan Province: Xishuangbanna, Menghai County, Bada Township, E 100°13’, N 21°14’, alt. 1900 m, associated with Castanopsis calathiformis, Ca. indica and Lithocarpus truncatus, 22 June 2020, G.S. Wang 960 (KUN-HKAS110157, GenBank Acc. No.: MZ412903 for ITS, MW413909 for nrLSU, MW439269 for tef1-a, and MW439262 for rpb2), the same location, 22 June 2020, G.S. Wang 965 (KUN-HKAS110158, GenBank Acc. No.: MZ412904 for ITS, MW439270 for nrLSU, MW439270 for rpb1, and MW439263 for rpb2); Nanjiang County, Gonglang Town, Huanggaoping, E 100°30’, N 24°54’, alt. 1200 m, associated with Castanopsis orthacantha, Lithocarpus mairei and Quercus griffithii, 30 June 2015, K. Zhao 773 (KUN-HKAS92401, GenBank Acc. No.: MZ536632 for nrLSU, MZ543307 for tef1-a, and MZ543309 for rpb2); Jinghong County, Dadugang Town, E 100°25’, N 21°26’, alt. 600 m, associated with Castanopsis indica and Lithocarpus truncatus, 30 June 2014, K. Zhao 476 (KUN-HKAS89139, GenBank Acc. No.: MZ536631 for nrLSU, MZ543306 for tef1-a, and MZ543308 for rpb2).

Commentary: Leccinum pseudoborneense is characterized by the nearly glabrous and pale brown to dark brown pileus, the white context in pileus not changing color when injured, the white context in stipe staining blue when bruised, the initially white and then brown hymenophore not changing color when injured, the white stipe covered with ochraceous to dark brown squamules, and the trichodermal pileipellis composed of 3–6 µm wide interwoven hyphae. Leccinum pseudoborneense is similar to L. borneense (Corner) E. Horak, originally described from Malaysia, in that they share a brown pileus, bluish color change of the context in stipe when bruised, and similar size of basidiospores. However, L. borneense differs from L. pseudoborneense in its yellow to olive yellow hymenophore staining blue when hurt, pale yellow to yellow pileal context staining blue when hurt, and deep yellow.
context in stipe staining blue but sometimes with reddish tint at base when injured [6,84]. *Leccinum pseudoborneense* is phylogenetically close to *L. album* in our phylogenetic analyses (Figures 1 and 2). However, *L. album* has a white basidioma, white hymenophore staining indistinctly greenish blue when hurt, white context in pileus not changing color when injured, white context in stipe unchanging or only staining distinctly greenish blue at base when injured, and relatively broad basidiospores measuring 15–19 × 5–7 µm.

4. Discussion

The genus *Leccinum* was defined and recognized variously by different mycologists. In an early molecular study, *Leccinum* was shown to be polyphyletic and proposed to be restricted to the sections *Leccinum* and *Scabra* by Binder and Besl [4]. Subsequently, Bresinsky and Besl [32] erected a genus *Leccinellum* Bresinsky & Manfr. Binder, to accommodate *L. section Luteoscapra*, including species with yellow hymenophores and/or context. In this study, the phylogenetic inferences based on the multi-locus dataset of nrLSU, tef1-α and rpβ2 largely coincide with those of Binder and Besl [4], Bresinsky and Besl [32] and den Bakker et al. [33]. Thus, we adopt the treatment of Bakker et al. [33] and treat *Leccinum* in a strict circumscription, which only includes species of *L. sect. Leccinum* (Singer’s infrageneric classification with *L. sect. Scabra* merged to this section). Species in *Leccinum* are characterized by the white context lacking color changes or staining blue, gray or reddish tints when injured and the cutis-like pileipellis composed of interwoven filamentous hyphae.

Eleven *Leccinum* species with specimen citations have been reported from China before this study, of which five species (*L. ambiguum*, *L. atrostipitatum*, *L. olivaceopallidum*, *L. potteri* and *L. subgranulosum*) were originally described from North America, five species (*L. aurantiacum*, *L. holopus*, *L. roseofructum*, *L. scabrum* and *L. versipelle*) were originally described from Europe, and only one taxon (*L. subleucophaeum var. minimum*) was originally described from China. Our molecular phylogenetic analyses along with morphological studies identified the existence of *L. quercinum*, *L. scabrum*, *L. subleucophaeum var. minimum* and *L. versipelle* in China. The distribution of other reported species have not yet been found, based on morphological and/or molecular data. In addition, three species new to science (*L. album*, *L. parascabrum* and *L. pseudoborneense*) and two species new to China (*L. melaneum* and *L. schistophilum*) were revealed in our study, based on molecular and morphology evidence. In conclusion, there are nine species of *Leccinum* in China.

Most species of *Leccinum* exhibit strong mycorrhizal host specificity. The host specificity along with climate type and edaphic factors appear to be important factors determining the distribution of different species. In China, *L. melaneum*, *L. scabrum*, *L. schistophilum* and *L. versipelle* are found in temperate forests and associated with plants of Betulaceae (*Castanopsis calathiformis*, *Ca. hystrix*, *Ca. indica*, *Ca. orthocantha*, *Cyclobalanopsis glauca*, *Lithocarpus mairei*, *Li. truncatus* and *Quercus griffithii* and/or Pinaceae (*Pinus kwangtudgensis* and *P. yunnanensis*) on acidic soils. It is noteworthy that *L. parascabrum* can be found in acidic or slightly alkaline habitats. *Leccinum versipelle* is found in subtropical forests and is associated with plants of *Populus yunnanensis* on acidic soils. The combination of the color of basidioma, the morphology of pileal surface, the size of basidiospores, the morphology of stipe, the color changes when injured, the climate type, the edaphic factors and the host preferences is very important in distinguishing species in this genus.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/jof7090732/s1, Alignment S1: alignment of multi-locus dataset; Alignment S2: alignment of ITS dataset.

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writing: X.M.; original draft—review and editing: Y.-C.L. All authors have read and agreed to the published version of the manuscript.

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