

Cortinarius subsalor and *C. tibeticisalor* spp. nov., two new species from the section *Delibuti* from China

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Abstract

Cortinarius subsalor and *C. tibeticisalor*, belonging to the section *Delibuti*, are described from China as new to science. *Cortinarius subsalor* has been found **to be** associated with *Lithocarpus* trees in subtropical China and resembling *C. salor*, but it differs **from** *C. salor* **in** the slender basidiomata and the narrower basidiospores, as well as **in** molecular sequence data. *Cortinarius tibeticisalor* were collected from eastern Tibetan Plateau, associated with *Abies*, **it** differs from other species **within** sect. *Delibuti* **in** the olive tinge of mature or dried basidiomata and bigger basidiospores. The molecular data also support *C. subsalor* and *C. tibeticisalor* as new species. The phylogenetic analyses and biogeography of sect. *Delibuti* are discussed and a key to the species of this section currently known in the world is provided.

Introduction

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43 | *Cortinarius* (Pers.) Gray is an ectomycorrhizal fungal genus, associated with a wide host range of
44 | plants, such as Betulaceae, Caesalpiniaceae, Cistaceae, Dipterocarpaceae, Fagaceae, Myrtaceae,
45 | Pinaceae, Rhamnaceae, Rosaceae, Salicaceae and some herbaceous plants (Frølev, Brandrud &
46 | Jeppesen, 2006; Niskanen, 2008). The genus is distributed worldwide. Even though it is the
47 | largest genus among macrofungi, but its species diversity is still unknown. Most of *Cortinarius*
48 | species were originally discovered from Europe and America, and rarely in Asia and Africa
49 | (Horak, 1983; Xie et al., 2020).

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50 | *Cortinarius* sect. *Delibuti* (Fr.) Sacc. with characteristics of viscid pileus and stipe, have
51 | usually been considered as a section in subg. *Myxaciium* (Fr.) Trog (Trog, 1844; Earle, 1902;
52 | Orton, 1955; Brandrud et al., 1989; Consiglio, Antonini & Antonini, 2003). *Delibuti* species can
53 | easily be distinguished by the anomaloid appearances, mild taste and subglobose basidiospores
54 | from other myxacioid species (Orton, 1955; Soop, 2014). Section *Delibuti* was also considered to
55 | belong to subg. *Phlegmacium* (Fr.) Trog (Bidaud, Moënne-Loccoz & Reumaux, 1992; Bidaud,
56 | Moënne-Loccoz & Reumaux, 1994). Recently, Soop et al. (2019) treated sect. *Delibuti* among
57 | anomaloid sections, not in myxacioid sections based on the shared characters of sect. *Delibuti*
58 | and sect. *Anomali* Konrad & Maubl., together with the close relationship in the phylogenetic
59 | analyses. In the past, numeral species were assigned to section *Delibuti* (Fries, 1838; Earle, 1902;
60 | Bidaud, Moënne-Loccoz & Reumaux, 1992; Soop, 2013; Soop, 2014), however most species have
61 | been confirmed not to belong to this section (Orton, 1955; Consiglio, 2012; Dima et al., 2016;
62 | Soop et al., 2019). Soop et al. (2019) defined only ten species in sect. *Delibuti*, but the
63 | phylogenetic analyses verified and showed that there can still be some undescribed species in this
64 | section (Harrower et al., 2011; Garnica et al., 2016; Soop et al., 2019). In China, four *Delibuti*
65 | species, *C. betulinus* J. Favre, *C. delibutus* Fr., *C. illibatus* Fr., and *C. salor* Fr., were reported
66 | (e.g. Teng, 1963; Yuan & Sun, 1995; Shao & Xiang, 1997; Li & Azbukina, 2011; Xie, 2018; Wang
67 | et al., 2020), but none of them have been confirmed by molecular sequence data, thus their
68 | occurrence in China is yet controversial.

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69 | We have conducted taxonomic and phylogenetic studies of *Cortinarius* in China. Several
70 | new *Cortinarius* species have been described from China (Wei & Yao, 2013; Xie et al., 2019; Xie
71 | et al., 2020; Xie et al., 2021; Yuan et al., 2020). Some glutinous violet *Cortinarius* specimens
72 | resembling *C. salor* were found during an intensive field work and later, during the identification
73 | process they turned out to be new species which we describe here based on morphological and
74 | ecological characteristics, as well as phylogenetic analyses evidences. We also discuss the
75 | phylogenetic relationship and biogeography of sect. *Delibuti*. An identification key to the known
76 | species of sect. *Delibuti* is provided.

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78 | Materials & Methods

79

80 | Specimens and morphological description

81 | Specimens were collected from Zhejiang Province and Tibet Autonomous Region, respectively.

82 | The collection sites in Zhejiang are the subtropical areas with the evergreen broadleaf forests

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dominated by *Lithocarpus brevipendulus*. Meanwhile, the collection sites in Tibet are the plateau-alpine areas with coniferous forests dominated by *Abies georgei* var. *smithii*. Fresh basidiomata were photographed in the field. Macroscopic characteristics were measured and recorded from every basidiomata, color codes follow *Kornerup & Wanscher (1978)*. Microscopic features were examined and described in 5% KOH, Congo Red or Melzer's reagent and observed under a Zeiss AX10 light microscope. Thirty to forty mature basidiospores were measured (excluding apiculus and ornamentation) per collection. The length/width ratio (Q) was calculated for individual basidiospores. X_{av} and Q_{av} refer to the average value of basidiospores of each specimen. Voucher specimens deposited in the Herbarium of Mycology, Jilin Agricultural University (HMJAU), Changchun, China.

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Phylogenetic reconstruction

DNA extraction, PCR amplifications, and sequencing methods followed *Xie et al. (2019)*. The primers ITS1F and ITS4 were used in both PCR and sequencing reactions for the nrDNA ITS region with (*White et al., 1990; Gardes & Bruns, 1993*). The newly generated ITS sequences were submitted to GenBank. The ITS sequences for the phylogenetic analyses were selected based on results of BLASTn (> 90% identity) in GenBank and UNITE and followed the publication by *Garnica et al. (2016)* and *Soop et al. (2019)*. Two species in section *Cyanites* Nespiak were chosen as outgroup followed *Xie et al. (2021)*.

Sequences (Table 1) for the phylogenetic analyses were aligned and edited with BioEdit 7.1.3.0 and Clustal X (*Tompson et al., 1997; Hall, 1999*). For phylogenetic analyses, Bayesian Inference (BI) and Maximum Likelihood (ML) methods were implemented in this study. MrModeltest 2.3 was used to calculate the best model (HKY+I+G) for Bayesian Inference (BI) analysis (*Nylander et al., 2008*). BI analysis was performed with MrBayes 3.2.6 (*Ronquist & Huelsenbeck, 2003*). Markov Chains Monte Carlo (MCMC) chains were run for 500 000 generations, sampling every 100th at which point the average standard deviation of split frequencies was 0.009032. A The 50% majority rule consensus tree of the BI trees sampled in the MCMC analyses and posterior probability values were estimated from the samples after discarding the first 25% (1250) of sampled trees. The ML analysis was performed with RAxML (*Stamatakis, 2014*) and implemented in raxmlGUI (*Silvestro & Michalak, 2012*). All parameters in the ML analysis were kept as defaults, except for choosing GTRGAMMA as the model of sequence evolution. For testing the support of the branches, rapid bootstrap analysis with 1,000 replicates was chosen. The phylogenetic trees were visualized in FigTree 1.4.3. The Bayesian posterior probabilities values (BPP) ≥ 0.95 and ML bootstrap values (ML) $\geq 75\%$ are shown on the branches at the nodes (BPP/ML).

Nomenclature

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work have been

submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix "http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

Results

Phylogenetic analyses

The alignment contained 67 ITS sequences with 785 nucleotide sites, include 260 informative sites (see Supplemental Material). The BI and ML trees showed similar topologies and ML tree was selected as the representative phylogeny (Figure 1). The phylogenetic analyses showed 11 sections including section *Cyanites* outgroup, in addition one singleton species from Argentina, and two singletons from New Zealand. Every section formed separate monophyletic lineages with strong statistical support. Section *Delibuti* formed a distinct clade (BPP = 0.96) separated from other sections. Section *Delibuti* split into five main clades based on the analyses of ITS sequences. Clade A and B consist of Australasian species. Clade C is a clade including our new species from the Tibetan Plateau. Clade D consist of the species distributed in Europe, Asia and North and South America. Clade E represents the species in the Northern Hemisphere. *Cortinarius subsalor* (BPP/ML = 1.00/100%, clade E) and *C. tibeticisalor* (BPP/ML = 1.00/100%, clade C) formed a distinct lineages with high statistical support, respectively. Furthermore, *C. subsalor* formed a sister relationship with the European *C. salor* (BPP = 0.97, clade E).

Taxonomy

***Cortinarius subsalor* M.L. Xie, T.Z. Wei & Y. Li, sp. nov.**

MycoBank No. MB839320

(Fig. 2)

Etymology. The name refers to the similar species *Cortinarius salor*.

Holotype. CHINA. Zhejiang: Baishanzu Mountain, Qingyuan county, on moist soil under *Lithocarpus brevicaudatus* (Fagaceae) forest with scattered Theaceae and *Rhododendron*, 27°45'44" N, 119°11'50" E, ASL 1510 m, 20 July 2020, Jun-Liang Chen, QY-0235(1192-1198) (HMJAU48759), GenBank: MW911734.

Diagnosis. Pileus hemispherical to plane, violet, glutinous; lamellae violet at first, then pale grayish violet; stipe slender, pale violet, then brown; glutinous veil violet. Basidiospores on average 8.0–8.3 × 6.9–7.0 μm, subglobose to broadly ellipsoid. Differing from other species in sect. *Delituti* by the violet color of basidiomata, the distribution of subtropical China and associat~~ion~~ with *Lithocarpus brevicaudatus*.

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Description

Pileus 20–50 mm, hemispherical at first, then convex to applanate; bluish violet (18B6-18C7) at first, purple (15B6-15C7) to purplish red (14A6-14B7) at the centre, then grayish violet (17B4-17C5), pale violet (19A3) at the margin; surface glutinous. Lamellae emarginate; moderately crowded; violet (17B6) when young, then grayish violet (17B4-17C5) to pale grayish violet (15B1-15C2); edge almost even. Stipe slender, 35–65 mm long, 3–7 mm thick, clavate at base (up to 14 mm); pale violet to grayish violet (19A3-19B5), later whitish, lightly brown to brown (7D6-7E7); surface with viscid universal veil, basal mycelium white with bluish tinge. Universal veil viscid, violet, remnants forming a girdle on the upper part of the stipe, disappearing with age. Context whitish at the pileus, slightly with yellowish tinge at the center, pale violet tinge extend outward, hygrophanous near lamellae; white with pale violet tinge at the apex of the stipe, yellow at the lower part; somewhat hollow within stipe. Odor not significant, taste mild.

Basidiospores $7.4\text{--}9.5\ (10.6) \times 6\text{--}7.7\ (8.7)\ \mu\text{m}$, $Q = 1.10\text{--}1.29$, $X_{av.} = 8.0\text{--}8.3 \times 6.9\text{--}7.0\ \mu\text{m}$, $Q_{av.} = 1.20$, subglobose to broadly ellipsoid, moderately coarsely verrucose, moderately dextrinoid. Basidia 4-spored. Lamellar edges fertile. Pileipellis: epicutis strongly gelatinous, about 180–250 μm thick, with hyphae 2–7 μm wide, yellowish to colorless in 5% KOH, with small encrusted spots, with some thick-walled hyphae. Hypodermium present; hypodermial hyphae 4–10 μm wide, cylindrical, almost colorless in 5% KOH, smooth. Clamp connections present.

Exsiccatae. Pileus grayish violet (19B3-19C4) at the margin, light brown to dark brown (6D6-6F8) at the centre; lamellae rust brown (6E8); stipe brown (6D7-6E7), lighter downwards, yellowish white (4A2) at base.

ITS sequence. The ITS sequence (MW911734, holotype) distinct from other members of sect. *Delibuti* and deviating from them by at least 22 substitutions and indel positions.

Ecology and distribution. In subtropical evergreen broadleaf forests, associated with *Lithocarpus brevicaudatus* (Fagaceae). Known from Zhejiang and Hunan province of China.

Additional specimens examined. CHINA. Zhejiang: Baishanzu Mountain, Qingyuan county, on moist soil under *Lithocarpus brevicaudatus* (Fagaceae) forest with scattered Theaceae and *Rhododendron*, 27°45'55" N, 119°11'0" E, ASL 1500 m, 20 August 2020, Meng-Le Xie, 20xml12101 (HMJAU48758), GenBank: MW911733.

***Cortinarius tibeticisolor* M.L. Xie, T.Z. Wei & Y. Li, sp. nov.**

MycoBank No. MB839321

(Fig. 3)

Etymology. The name refers to the Tibetan Plateau, the type locality, and the similarity with *C. salor*.

Holotype. CHINA. Tibet Autonomous Region: Sejila Mountain, Linzhi city, on moist soil in *Abies* forest with scattered *Rhododendron*, 29°35'26" N, 94°35'53" E, ASL 4120 m, 5 September 2020, Meng-Le Xie, 20xml12416 (HMJAU48764), GenBank: MW911729.

Diagnosis. Pileus hemispherical to applanate, violet, glutinous, margin wavy, somewhat olive when mature; lamellae for a long time violet, then pale grayish violet to violet gray; stipe

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218 | robust, bluish gray to brown with olive tinge; veil glutinous, violet. Basidiospores on average
219 | 10.3–10.8 × 8.7–8.9 μm, subglobose to broadly ellipsoid, rarely ellipsoid. Differing from other
220 | species in sect. *Delituti* by the olive tinge of basidiomata and the large basidiospores.

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221 | **Description**

222 | Pileus 50–85 mm, hemispherical at first, then convex to plane, sometimes slightly depressed,
223 | wavy at margin of mature basidiomata, violet (17C7) at first, especially at the centre, paler violet
224 | towards the margin, then grayish orange (5B5) to brown (5D6–5E7) with olive tinge, dark at the
225 | centre; surface glutinous. Lamellae emarginate, moderately crowded, persistently violet (17C7),
226 | then grayish violet (19B4–19C6) to violet gray (19B2); edge uneven, slightly serrate. Stipe 85–
227 | 120 mm long, 10–15 mm thick, clavate at base (up to 23 mm); surface with viscid bluish gray
228 | (19B2) universal veil remnants, then becoming yellow to brown with olive tinge (4B6–4D7),
229 | grayish violet (19B4–19C6) at the apex; basal mycelium white. Universal veil viscid, violet,
230 | remnants forming a girdle on the upper part of the stipe, disappearing with age. Context white with
231 | marbled violet tinge at first, slightly yellowish from the center of the pileus, then yellow at the
232 | stipe, especially at the middle. Odor weak, when fresh, somewhat like honey when old or dry.
233 | Taste mild.

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234 | Basidiospores 9.0–12.0 (13.5) × 7.7–10.6 μm, Q = 1.10–1.39, X_{av.} = 10.3–10.8 × 8.7–8.9
235 | μm, Q_{av.} = 1.20–1.23, subglobose to broadly ellipsoid, rarely ellipsoid, moderately coarsely
236 | verrucose, weakly dextrinoid. Basidia 4-spored. Lamellar edges fertile, with narrow clavate cells.
237 | Pileipellis: epicutis strongly gelatinous, about 300–410 μm thick, hyphae 3–8 μm wide, with
238 | yellowish intracellular pigment in 5% KOH, smooth. Hypodermium present, hyphae 7–15 μm
239 | wide, irregular, almost colorless in 5% KOH, smooth. Clamp connections present.

240 | **Exsiccatae.** Pileus olive brown (4E6–4F7) at margin, yellowish brown (5D7–5E8) at centre;
241 | lamellae dark bluish gray (19E2–19F2); stipe bluish white at apex, light brown (6D6–6E7) to dark
242 | brown (6F4–6F8), white at base.

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243 | **ITS sequence.** The ITS sequence (MW911729, holotype) distinct from other members of
244 | sect. *Delibuti* and deviating from them by at least 40 substitutions and indel positions.

245 | **Ecology and distribution.** In plateau-alpine coniferous forests, associated with *Abies*
246 | (Pinaceae) trees. Known from Tibetan Plateau of China.

247 | **Additional specimens examined.** CHINA. Tibet Autonomous Region: Sejila Mountain,
248 | Linzhi city, on moist soil under *Abies* forest with scattered *Rhododendron*, 29°35'25" N,
249 | 94°35'55" E, ASL 4170 m, 28 August 2019, Meng-Le Xie, 19xml10976 (HMJAU48761),
250 | GenBank MW911731, 19xml10981 (HMJAU48762), GenBank MW911732; Sejila Mountain,
251 | Linzhi city, on moist soil under *Abies* forest with scattered *Rhododendron*, 29°35'26" N,
252 | 94°35'53" E, ASL 4120 m, 5 September 2020, Meng-Le Xie, 20xml12395 (HMJAU48763),
253 | GenBank: MW911730.

254 |
255 | **Key to species of sect. *Delibuti***
256 | 1 Distributed in Northern Hemisphere 2
257 | - Distributed in Southern Hemisphere 9
258 | 2. Pileus usually yellowish to ochraceous without blue 3

批注 [12]: Please carefully double check the characteristics of each species not to contradict the description in the Key.

273	- Pileus more or less violet to blue when young , sometimes partly yellow,.....	4
274	3. Lamellae usually blue when young, veil yellowish	C. delibutus
275	- Lamellae pinkish ochraceous clay, veil not yellowish	C. illibatus
276	4. Pileus frankly blue when young, stipe bluish, veil violet	5
277	- Pileus grayish blue to olive brown , stipe pale, veil different,.....	7
278	5. Basidiomata usually small, lamellae violet, then grayish to brownish, stipe usually slender (<	
279	10 mm), base white with bluish tinge, basidiospores on average 8.0–8.3 × 6.9–7.0 μm,	
280	subglobose to broadly ellipsoid, distributed in subtropical China, associated with <i>Lithocarpus</i>	
281	<i>brevicaudatus</i>	C. subsalor
282	- Basidiomata usually bigger, lamellae persistently lilaceous or bluish, stipe usually more robust (>	
283	10 mm thick)	6
284	6. Pileus usually staining buff or fading from the centre, stipe base usually grayish brown,	
285	basidiospores 7–9 × 6–8 μm, globose to subglobose, distributed in Europue, associated with	
286	deciduous and coniferous trees	C. salor
287	- Pileus usually olive brown when mature, stipe base usually white, basidiospores 10.3–10.8 ×	
288	8.7–8.9 μm, subglobose to broadly ellipsoid, rarely ellipsoid, distributed in Tibetan Plateau of	
289	China, associated with <i>Abies</i>	C. tibeticisalor
290	7. Basidiomata small, pileus yellow to olive-ochre at the centre, grayish blue towards the margin,	
291	soon fading, veil yellow, basidiospores 7.5–9.5 × 6.5–7.5 μm, subglobose, associated with	
292	<i>Betula</i>	C. betulinus
293	- Basidiomata robust, associated with <i>Picea</i>	8
294	8. Pileus olive brown with a violet margin, veil olive brown, basidiospores 8–10 × 7–8	
295	μm,	C. transiens
296	- Pileus more orange tinge, basidiospores 7.5–9.5 × 6.5–7.5 μm,	C. largodelibutus
297	9. Associated with <i>Nothofagus</i>	10
298	- Associated with Myrtaceae trees	11
299	10. Pileus viscid, blue-green to aerugineous, stipe blue green, basidiospores 6.5–8.5 × 6–7 μm,	
300	subglobose, destributed in Australasia	C. tessiae
301	- Pileus glutinous, greyish yellow to greyish orange, stipe violet, then becoming white to pale	
302	brownish, basidiospores ellipsoid, destributed in North and South America	C. illitus
303	11. Basidiomata distinctly viscid to glutinous, mainly greyish blue-green, basidiospores 7–9 × 7–	
304	8 μm, globose to subglobose	C. rotundisporus
305	- Basidiomata weakly viscid, stipe often dry, mainly yellow-green to olive, Veil orange to	
306	ochraceous, basidiospores 6–7.5 × 5.5–6.5 μm, subglobose	C. calaisopus

307

308 **Discussion**

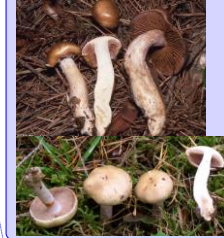
309 *Cortinarius subsalor* is similar with *C. betulinus*, *C. salor* and *C. transiens* (Melot) Soop due to

310 the bluish tinge of the basiodiomata. However, *C. betulinus* is usually grayish blue at the margin

311 of pileus and soon fading, the stipe **is** often pale and the veil usually is yellow (Kibby, 2005;

312 *Niskanen et al., 2008; Soop, 2014*). The pileus of *C. transiens* is often with a violet tone towards

批注 [13]: Some species are not so obviously "violet to blue", for example: *C. largodelibutus*.



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批注 [14]: Some species are not so obviously "grayish blue to olive brown", for example: *C. largodelibutus*.



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批注 [15]: The spore sizes and subglobose forms are so close, that it is suggested to add an additional comparative character here.

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321 the margin, while the centre is more olive gray to yellowish brown even in young specimens, the
322 | stipe is pale, and the gelatinous veil is olive brown (Soop, 1990; Soop, 2014). In China,
323 sometimes some bluish myxacioid species have been misidentified as *C. salor* (MHHNU30409,
324 GenBank: MK250915), collected from Hunan Province. Our phylogenetic analyses showed that
325 this sequence belong to the new species *C. subsalor*. *Cortinarius salor* has persistently lilaceous
326 | lamellae, the stipe is more robust (> 10 mm thick) and the base is more grayish brown, the the
327 basidiospores are rounder (7–9 × 6–8 μm), and it occurs in European woodlands (Orton, 1955;
328 *Consiglio*, *Antonini & Antonini*, 2003; Soop, 2014). Based on these features, *C. salor* can be
329 distinguished from the Asian *C. subsalor*.

批注 [I6]: "As well as" is somewhat different from "and".

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330 *Cortinarius tibeticisalor* is characterized by the basidiomata usually violet when young, then
331 | grayish orange to brown with an olive tinge, larger basidiospores and a restricted distribution in
332 the Tibetan Plateau. *Cortinarius tibeticisalor* is similar to *C. salor* in young stage, however, the
333 | basidiospores (7–9 × 6–8 μm) of *C. salor* are significantly smaller and rounder, and the
334 basidiomata never have olive tinge (Orton, 1955; *Consiglio*, *Antonini & Antonini*, 2003; Soop,
335 2014).

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336 According to our phylogenetic analyses, sect. *Delibuti* demonstrates a widely distributed
337 lineage of *Cortinarius*, in both the Northern and Southern Hemispheres. This bihemispherical
338 distribution is also seen in several other lineages in *Cortinarius*, such as *Anomali*, *Bolares*,
339 *Camphorati*, *Defibulati*, *Illumini*, and *Vibratiles*, which result is in concordance with other papers
340 (e.g. Harrower et al., 2015; Garnica et al., 2016; Soop et al., 2019). The nrDNA ITS region is
341 not suitable to draw conclusions for comprehensive phylogenetic evaluation, however, there are
342 some interesting patterns indicated in sect. *Delibuti* to be further discussed. The basal lineages
343 | (clade A and B) of *Delibuti* are solely distributed in the Australasia, showing a presumable origin
344 of the section in Australasia. Interestingly, the clade D contains species from multiple continents
345 in the Northern and Southern Hemispheres. Some species distributed in Asia (*Cortinarius* sp.,
346 LC175538), in Europe (*Cortinarius* sp., JF907917), and in South America, like *Cortinarius* sp.
347 (MF599228) from Colombia and *C. illitus* M.M. Moser & E. Horak, originally described from
348 Argentina (Moser & Horak, 1975), but also found in North America (according to the sequences
349 in GenBank). These patterns could explain that the evolution of sect. *Delibuti* is limited to the
350 ectomycorrhizal host specificity, as well as geographic barriers (Wang & Qiu, 2006; Brandrud,
351 1996; Wilson et al., 2017; Feng et al., 2016). The evolution and origin of sect. *Delibuti*, even of
352 genus *Cortinarius* will be a subject for future research.

批注 [I7]: Grammatically incorrect

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