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Cortinarius jiaoheensis (Cortinariaceae), a new species of *Cortinarius* subgenus *Telamonia* section *Flexipedes*, from northeast China

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Abstract

A new *Cortinarius* species in subgenus *Telamonia* section *Flexipedes*, *Cortinarius jiaoheensis*, is described based on morphological characteristics and molecular data. It is characterized by small basidiomata, with the surface of the pileus completely covered by woolly squamules, and ellipsoid to obovoid ellipsoid basidiospores. This species produces basidiomata in the autumn and is currently known only from northeast China.

Keywords: Agarics, ectomycorrhizal, ITS, SEM, taxonomy

Introduction

Cortinarius (Pers.) Gray. (1821: 627), the largest genus of Agaricales with about 2250 species distributed worldwide (He *et al.* 2019), is an important ectomycorrhizal genus in several ecosystems (Singer 1986). To date, over 5000 names and combinations have been published (Index Fungorum, http://www.indexfungorum.org, 15 November 2020 release).

Over the past ca. 200 years, several scientists have attempted to describe and classify *Cortinarius*. Persoon (1801) first described *Cortinarius* as a section under *Agaricus*, which included 52 species. Gray (1821) separated it from *Agaricus* as an independent genus. Fries (1838) laid the foundation for classification of *Cortinarius* by reporting 216 species of *Cortinarius* in Europe and dividing them into six subgenera based on the characteristics of pileus, stipe, and universal veil *viz*., *Myxacium*, *Phlegmacium*, *Inoloma*, *Dermocybe*, *Telamonia*, and *Hydrocybe*. Orton (1958) merged *Telamonia* into *Hydrocybe*, and split *Inoloma* into *Cortinarius* and *Sericeocybe*. Moser and Horak (1975) divided *Cortinarius* into nine subgenera, separated *Telamonia* from *Hydrocybe* and categorized it as a separate subgenus. Niskanen *et al.* (2015) divided *Cortinarius* into nine subgenera: *Telamonia, Myxacium*, *Phlegmacium*, *Dermocybe*, *Cortinarius*, *Orellani, Callistei, Camphorati,* and *Illumini*. Soop *et al.* (2019) identified 37 previously described sections and 42 sections described as new or based on taxa previously at a different rank.

C. subgenus *Telamonia* (Fr.) Trog (1844: 43), the most diverse and taxonomically challenging subgenus in *Cortinarius*, has most of its species occurring in the Northern Hemisphere (Niskanen 2007; Niskanen *et al.* 2008; Dima *et al.* 2014; Soop *et al*. 2019; Kokkonen *et al*. 2020), and some in the Southern Hemisphere (Peintner *et al.* 2003; Garnica *et al.* 2005). Telamonioid species are characterized by a dry stipe and dry or fairly dry, and in most species, ±hygrophanous pileus. The universal veil is white or colorful. In many species the basidiomata are without bright colors. The size of the basidiomata varies greatly from very small and slender to big and fleshy. Subgenus *Telamonia*, section *Flexipedes* Kytöv., Niskanen & Liimat. (2020: 1) contains species with small to very small basidiomata that have a smooth or scaly, brown pileus, with purplish tints in some species, a white universal veil that forms complete and incomplete girdles on the stipe or is sparsely distributed, and often a pelargonium-like or fragrant odour of the lamellae. Basidiospores are ellipsoid, ellipsoid-fusiform to narrowly fusiform. Lamellar trama and pileipellis hyphae are usually distinctly encrusted (Niskanen *et al*. 2020).

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Despite extensive research on *Cortinarius* in other parts of the world, the study and knowledge in China of this genus is limited. The reports are not properly documented in one place, most appearing in reference books, local atlases, and journals. Previous research on *Cortinarius* in China is based on the early classification method by Fries (1838). Out of more than 170 species of *Cortinarius* reported in China, subgenus *Telamonia* lacks extensive studies, with only about 30 species reported or described so far (Li *et al.* 2015).

In this investigation, we studied this new species of *Cortinarius* by integrating morphological and phylogenetic analyses and comparing it with similar species of *Cortinarius*.

Materials and methods

Study site and specimen collection

The specimens were collected from Jiaohe County, Jilin Province, Northeast China (43°55′19″N, 127°05′17″E, elev. 483 m), during fungal survey of the region. The annual mean temperature in this region ranges between -2.1 ℃ and 2.6 ℃, and the annual precipitation is between 510 mm and 810 mm. The main tree species distribution is the north temperate zone distribution type and includes mainly *Pinus koraiensis*, *Abies holophylla*, *Acer mandshuricum* Maxim., *Acer mono* Maxim., *Betula platyphylla*, *Carpinus cordata*, *Fraxinus mandshurica*, *Juglans mandshurica* Maxim., *Maackia amurensis*, *Quercus mongolica*, *Tilia amurensis*, and *Ulmus laciniata*. The specimens were found growing in broadleaved forests near *Quercus mongolica*. They were dried in silica gel and preserved in the Herbarium of Mycology of Jilin Agricultural University (HMJAU).

Morphological analysis

Fresh specimens were photographed in the field and macroscopic features were recorded. Dried specimens were used to observe microscopic features. The specimens were prepared by mounting hand-cut sections of the basidiomata in 5% (w/v) KOH and staining with Congo Red as required. The features were then observed using an 80i light microscope (Nikon, Tokyo, Japan). At least 20 mature basidiospores (n = 20) were examined from each fruitbody. The sterigmata of the basidia and the ornamentation of basidiospores were not included in the spore measurements. The ornamentation of the spores was observed by scanning electron microscope (SEM; (XL-30 ESEM FEG, FEI COMPANY™). The Methuen Handbook of Color (Kornerup & Wanscher 1978) was used to determine color codes. Flora Agaricina Neerlandica, Volume 1 (Bas *et al*. 1988) was followed for morphological terminology. Abbreviations are as follow: 'L' refers to the number of lamellae reaching the stipe; 'I' refers to the number of lamellulae between each pair of lamellae; 'Q' refers to the length / width ratio of basidiospores.

Phylogenetic analysis

Genomic DNA was extracted from 0.1 to 0.2 mg of dried specimen using a NuClean Plant Genomic DNA kit (CWBIO, Beijing, China) and preserved at -20°C. The internal transcribed spacer (ITS) region was amplified with the PCR primer pair ITS1/ITS4 (White *et al.* 1990; Gardes & Bruns 1993) using a touchdown cycling program (Don *et al.* 1991). For the molecular phylogenetic analysis, a total of 48 sequences were downloaded from GenBank, including closely related species and outgroups. Two species of section *Dermocybe* Pers., *C. birkebakii* Harrower Ammirati, Niskanen & Liimat (2014: 196) and *C. neosanguineus* Ammirati, Liimat & Niskanen (2013: 352,) were selected as outgroups based on the phylogeny by Stensrud *et al.* (2014). Detailed sequence information is given in Table 1. The ITS sequences newly generated from the Chinese samples have been deposited in GenBank (Accession nos. MW263943–MW263945). The full-length sequences were aligned using BioEdit v7.1.3 (Hall 1999). The phylogenetic tree was constructed with MrBayes v3.2.6 using the best-fit model (GTR+I+G) selected by AIC in MrModeltest v2.3 (Posada *et al.* 1998). Bootstrap analyses with the Randomized Axelerated Maximum Likelihood (RAxML) program were implemented in raxmlGUI1.5b1 (Stamatakis *et al.* 2005). Finally, FigTree v1.4.3 was used to visualize the phylogenetic trees. The overall results were expressed as a graphic phylogeny.

TABLE 1. Specimens used in the phylogenetic analysis and GenBank accession numbers. The new species is in bold.

Species		Locality	Voucher	GenBank accession number
1	*Cortinarius 'acutus'*	UK	UBC: F17138 OC66	GQ159881
2	*C. ammophilus*	UK	K:109694	MT934867
3	*C. andreae*	USA	H:T. Niskanen 07-392	MT934871
4	*C. birkebakii*	Canada	OC17	FJ039593
5	*C. birkebakii*	USA	WTU:Birkebak 10-20-2007-18	KP087973
6	*C. castaneopallidus*	France	PC:2001-124	MT934952
7	*C. desertorum*	Czech Republic	PRM:154750	MT935008
8	*C. diasemospermus*	Switzerland	288/17	MN841067
9	*C. diasemospermus*	Norway	312/16	MN841066
10	*C. difficillimus*	France	PC:97.12.28.08	MT935013
11	*C. flabellus*	Sweden	S:H. Lindstrom CFP672	MT935053
12	*C. flexipes*	Sweden	S:H. Lindstrom CFP802	MT935061
13	*C. flexipes*	Finland	547/16	MN841059
14	*C. flexipes*	UK	UBC F17187 OC115	GQ159777
15	*C. fragrantissimus*	USA	WTU:M.Beug 10MWB111913	KU041739
16	*C. fragrantissimus*	USA	WTU:M.Beug 10MWB111913	NR153058
17	*C. friesianus*	France	PC:97112425	MT935065
18	*C. 'fulvescens'*	--	PK5750	HQ604731
19	*C. 'fulvescens'*	UK	UBC F17171 OC99	GQ159914
20	*C. geraniolens*	France	PC:A. Bidaud 95-09-88	MT935093
21	*C. geraniolens*	Spain	JDRG23111205	MK696090
22	*C. goniosporus*	France	PC:2001-138	MT935098
23	*C. heterosporus*	Finland	H:T. Niskanen 05-162	MT935128
24	*C. impolitus*	USA	MICH:10366	MT935152
25	*C. jiaoheensis*	China	HMJAU56920_1	MW263943
26	*C. jiaoheensis*	China	HMJAU56920_2	MW263944
27	*C. jiaoheensis*	China	HMJAU56920_3	MW263945
25	*C. lindstroemii*	Sweden	S:H. Lindstrom CFP690	MT935198
26	*C. megacystidiosus*	France	PC:2009-81	MT935217
27	*C. megacystidiosus*	France	PC:2009-81	MT935217
28	*C. neosanguineus*	Canada	F14888	FJ157089
29	*C. neosanguineus*	USA	TN09-130	JX045678
30	*C. paleaceus*	Canada	PK4550	FJ039709
31	*C. pelargoniostriatulus*	USA	H:T. Niskanen 09-080	MT935292
32	*C. pelargoniostriatulus*	France	PC:A. Bidaud 07-08-42	MT935291
33	*C. pertristis*	Switzerland	G:13308/161	MT935295
34	*C. pilatii*	Czech Republic	PRM:655887	MT935305
35	*C. pilatii*	Sweden	S:H. Lindstrom CFP657	MT935306
36	*C. pinisquamulosus*	Sweden	S:H. Lindstrom CFP984	MT935307
37	*C. quercoconicus*	Sweden	H:7018166	MG136825
38	*C. rusticellus*	Switzerland	G:13512/165a	MT935405
39	*C. rusticellus*	Finland	H:6029429	MT935406

.....continued on the next page

TABLE 1. (Continued)

Species		Locality	Voucher	GenBank accession number
40	*C. subdepressus*	France	PC:4705	MT935476
41	*C. 'velenovskyi'*	UK	UBC F17172 OC100	GQ159762
42	*C. violaceopapillatus*	Norway	H:I. Kytovuori 11-020	MT935296
43	*C. violaceopapillatus*	France	PC:A. Bidaud 06-10-196	MT935153
44	*C. violaceopapillatus*	France	GE17.014	MT452497
45	*C. vulpicolor*	USA	IB:M. Moser 83/355	MT934906
46	*C. vulpicolor*	USA	1983/355	MN841041
47	*C. vulpicolor*	Switzerland	G:13282/148a	MT935097
48	*C. vulpicolor*	France	PC:A. Bidaud 91-08-51	MT935309

Results

Phylogenetic analysis

After the exclusion of areas with ambiguous alignment, 619 positions were used for analysis. The Bayesian Analysis (BA) and Maximum Likelihood (ML) trees (Fig. 1) show the same phylogenetic topology, with *C. jiaoheensis* as a new species that belongs to genus *Cortinarius*, subgenus *Telamonia*, section *Flexipedes*. The position of the species of *Cortinarius*, except for *C. jiaoheensis,* agrees with previous studies (Harrower *et al.* 2011; Liimatainen *et al.* 2020).

The phylogenetic trees show that *C. jiaoheensis* was strongly supported in our phylogenetic analysis (PP = 1.00, ML = 100) and grouped with *C. vulpicolor* but with a low support (PP = 0.50, ML = 65, group marked as Clade 1 in Fig. 1). The interspecific difference between *C. jiaoheensis* (MW263943–MW263945) and *C. vulpicolor* (MN841041, MT934906) is > 2.3% (14 substitutions and indels).

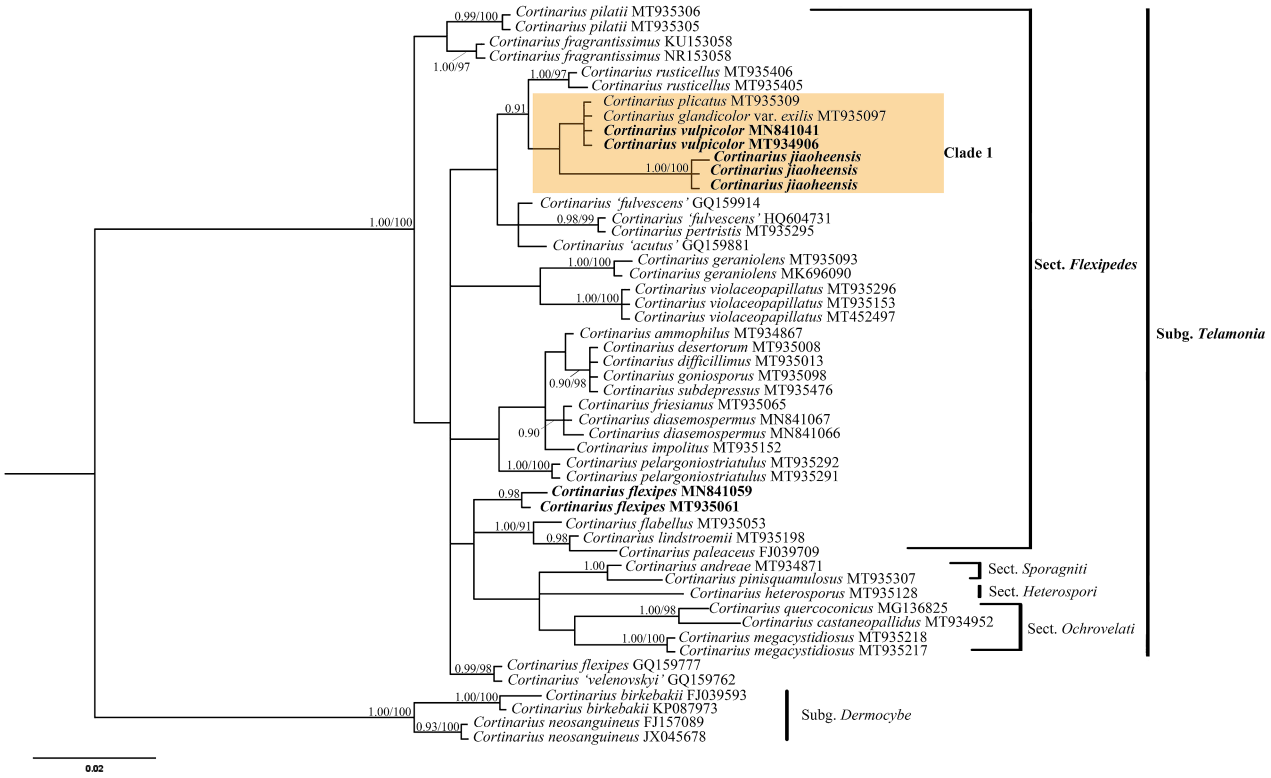

FIGURE 1. MrBayes and RAxML phylogram inferred from partial ITS sequence data. The tree is rooted with *Cortinarius birkebakii* and *C. neosanguineus*. Posterior probalities (>0.90) and maximum likelihood support values (>90) are showed on each branch (PP/MP). The new species is in bold.

Taxonomy

Cortinarius jiaoheensis T. Bau & Y. Luo *sp. nov*. (Figs.2–4)
MycoBank number:—MB838067

FIGURE 2. *Cortinarius jiaoheensis* (HMJAU56920, holotype!) Bars: A–C= 0.5 cm. Photos by Tolgor Bau.

Diagnosis:—Basidiomata small. Pileus 6–12 mm diam, at first conical later almost plane with a prominent umbo, surface completely covered by woolly, whitish squamules. Lamellae emarginate, leather brown(6E6). Stipe slender, cylindrical, surface covered by white fibrils, greyish brown underneath, with rich white mycelium at the base. Universal veil white, forming zones on the stipe. Odor in context indistinct, not observed in lamellae. Taste somewhat spicy. Basidiospores 7.8–9.2 × 5.8–6.8 µm, broadly ellipsoid to somewhat obovoid ellipsoid, light brown (6D8) to rust brown (6E8), verrucose. Basidia with (2) 4 sterigmata. Lamellae margin with sterile cells.

Etymology:—'jiaoheensis' refers to its occurrence in Jiaohe County, Jilin Province, China.

Holotype:—CHINA. Jilin Province: Shansong ridge, Jiaohe County, Jilin City, 43°55′19″N, 127°05′17″E, elev. 483 m, *Tolgor Bau*, 3 September 2018 (HMJAU56920!).

Description:—*Basidiomata* small. *Pileus* 6–12 mm diam, conical when young, later almost applanate to applanate with a prominent umbo, margin undulating; margin initially incurved, then slightly applanate to sometimes upturned; surface greyish brown (5D3–5E3) but completely covered by woolly, greyish to whitish squamules making the overall appearance pale, squamules flattened to slightly erect, dense at centre, becoming sparser and extending to the edge of the pileus. *Lamellae* moderately broad, with three lamellulae, light brown (6D6) when young, later leather brown (6E6), margin uneven, L=23–28, I=1–3. *Stipe* 17–26 mm long, 1–2 mm thick, central, cylindrical and hollow, equal in width, fragile, greyish brown (5E3), surface covered with white fibrils, with copious white mycelium at the base.

Universal veil white, forming zones on the stipe. *Context* thin, pale white (4A1) to yellowish grey (4B2), unchanging upon cutting. *Odor* in context indistinct, not observed in lamellae. Taste slightly spicy.

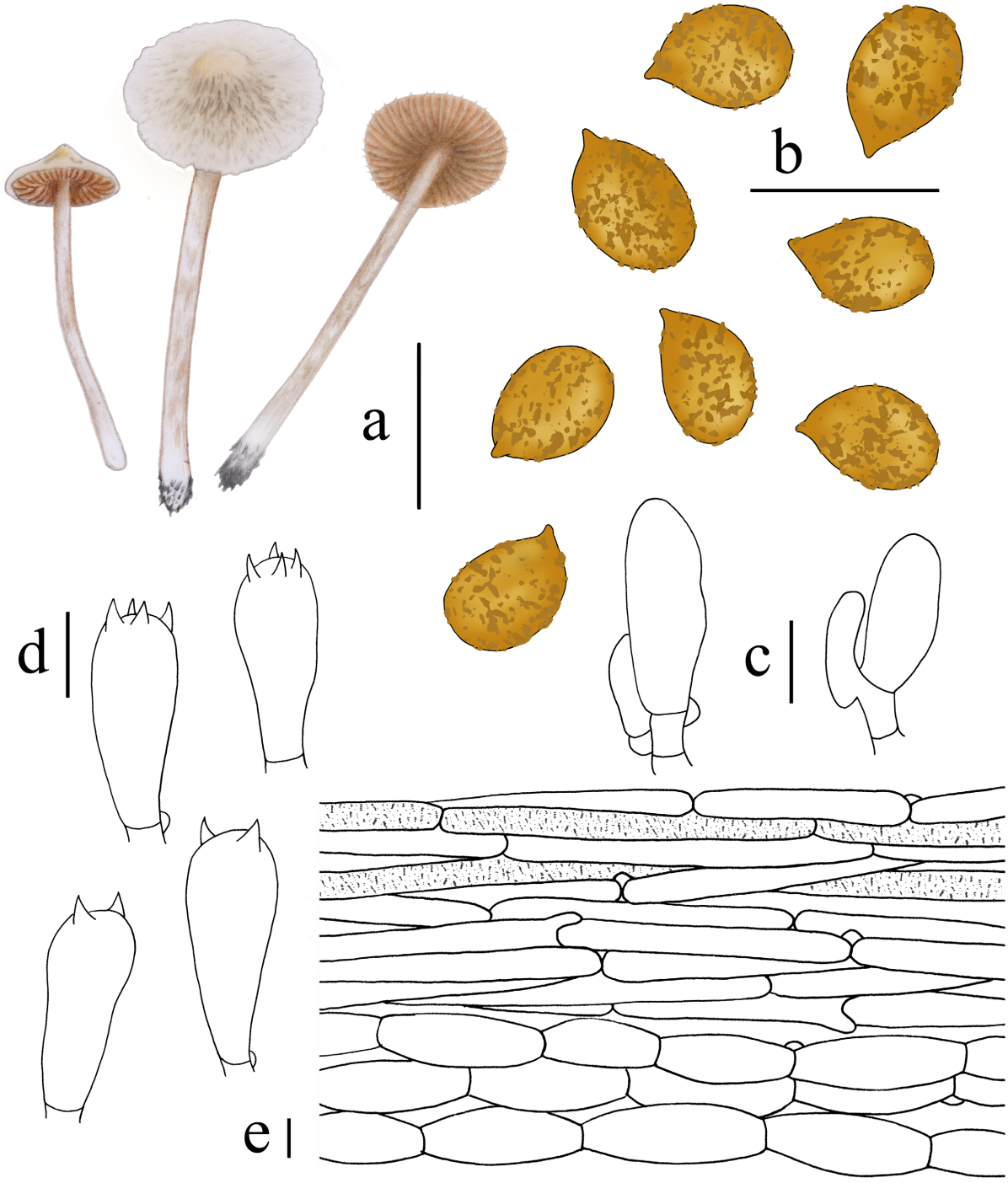

FIGURE 3. *Cortinarius jiaoheensis* (HMJAU56920) a. Basidiomata; b. Basidiospores; c. Marginal cells; d. Basidia; e. Pileipellis. Bars: a = 1 cm; b–h =10 µm. Drawings by Ying Luo.

Basidiospores 7.8–9.2 × 5.8–6.8 µm (n=20), Q=1.23–1.50, Qav=1.37, broadly ellipsoid to somewhat obovoid-ellipsoid, light brown (6D8) to rust brown (6E8), verrucose. *Basidia* 24.3–29.2 × 9.7–11.7 µm, clavate, thin-walled, with two or four sterigmata, colorless to sometimes yellowish. *Lamella margin* with sterile cells, 19.5–27.3 × 7.8–9.8 µm, clavate, colorless. *Pileipellis* an epicutis of hyphae 6–13 µm wide, cylindrical, colorless to sometimes yellowish, some encrusted; hypoderm well developed, elements 15–25 µm wide, thin-walled, hyaline, subcellular; clamp connections present.

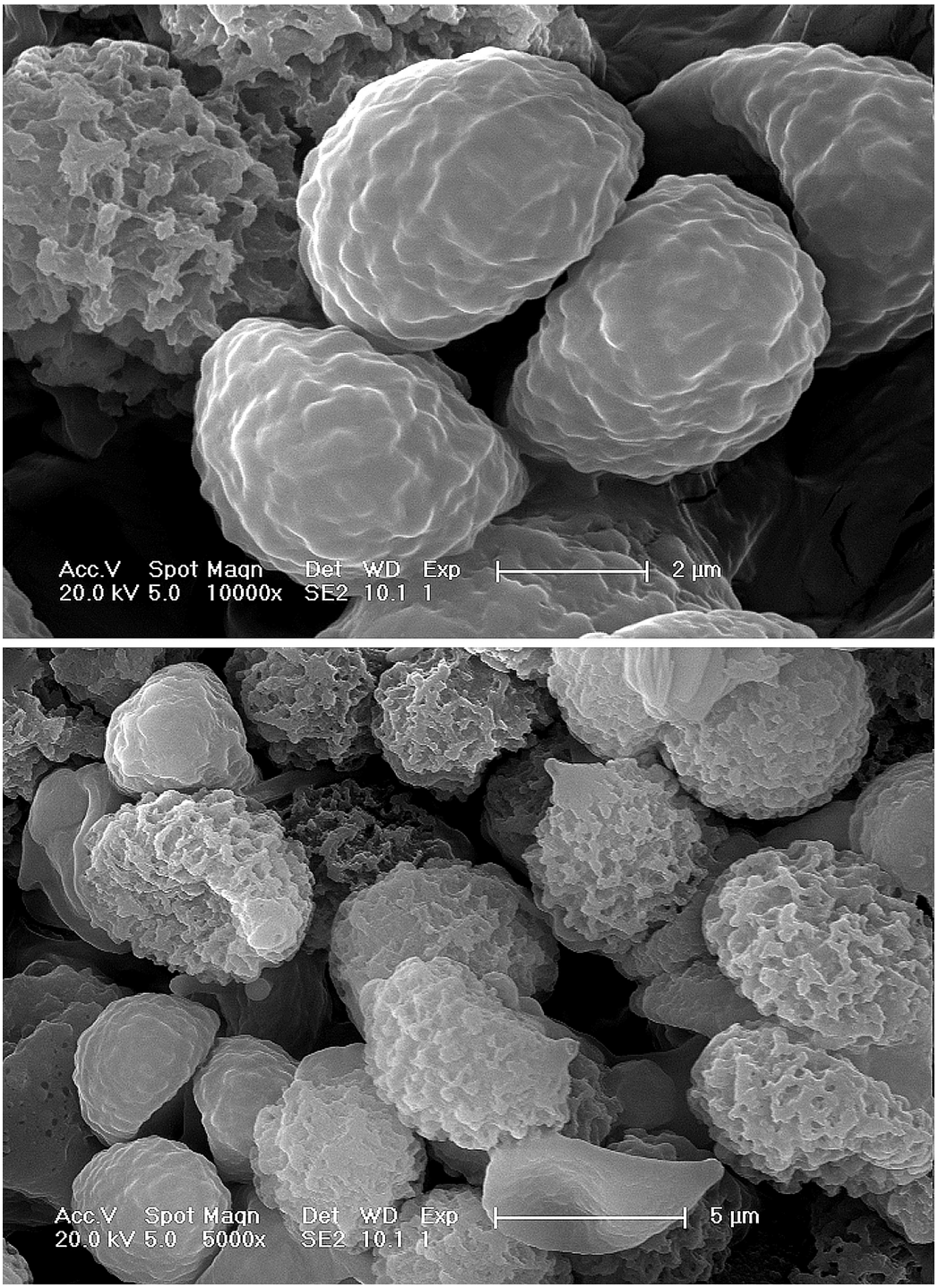

FIGURE 4. Scanning electron micrographs of *Cortinarius jiaoheensis* (from holotype, HMJAU56920) showing verrucose basidiospores. Photos by Ying Luo.

Habitat:—Scattered in broadleaved forests near *Quercus mongolica*, fruiting in autumn.

Known distribution:—Jilin Province, China.

Additional material examined:—CHINA. Jilin Province: Shansong ridge, Jiaohe County, Jilin City, 43°55′19″N, 127°05′17″E, elev. 483 m, *Tolgor Bau*, 3 September 2018, HMJAU56920.

Discussion

Based on morphological and phylogenetic characteristics, *Cortinarius jiaoheensis* is a well-defined species and

considered new in genus *Cortinarius*. From interpretations based on the first extensive phylogenetic study of the subgenus (Liimatainen *et al.* 2020) and the phylogenetic tree based on ITS (Fig. 1), we infer that *C. jiaoheensis* belongs to subgenus *Telamonia*, section *Flexipedes*. Furthermore, our investigation showed that the morphological description of *C. jiaoheensis* agrees with the previous reports of the species of section *Flexipedes* (Niskanen *et al*. 2020).

Cortinarius jiaoheensis is closely related to *C. vulpicolor*; the interspecific difference between the species is > 2.3% in the ITS region. Morphologically *C. vulpicolor* differs from *C. jiaoheensis* in having somewhat larger basidiomata (pileus up to 2.5 cm, stipe up to 6 cm long and 0.4 cm wide), an only fibrillose, not distinctly squamulose pileus, and relatively narrower basidiospores (7.5–10 × 5–6.5 µm). The habitats of the two species are also different: *C. jiaoheensis* is scattered in broad-leaved forests of *Quercus mongolica*, while *C. vulpicolor* grows with *Salix* (*herbacea*-like) but has also been found in *Picea*-dominated forests.

Both *C. vulpicolor*, and *C. flexipes* (Pers.) (Fries 1838) from the same section display some macromorphological similarities with *C. jiaoheensis*. They share a white fibrillose pileus and stipe, but the squamulosity of the pileus is denser in *C. jiaoheensis* than in *C. flexipes* and the basidiomata are smaller. The fibrous brown stipe of *C. flexipes* has more distinct white zones left by the universal veil than *C. jiaoheensis*. Furthermore, *C. flexipes* differs in the distinct pelargonium-like smell. Despite some similarities in macromorphological features, the phylogenetic relationship between the two species is relatively distant (Fig. 1).

Cortinarius jiaoheensis is currently known only from northeast China. Further collecting could extend the range and find more species of *Cortinarius* in this huge country.

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