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Phylogeny and taxonomy of *Climacocystis* (Polyporales) in China

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Abstract – *Climacocystis* was a monotypic genus typified by *C. borealis*. During a continuous survey of poroid basidiomycetes over China, several specimens of a *Climacocystis* species were collected at high elevations in southwestern China. They deviated from the type species, *C. borealis* in several microscopic features, including larger and ellipsoid to subcilindrical basidiospores (6-8.8 × 3-4.2 µm versus 5-6.8 × 3.2-4 µm), smooth cystidia (incrusted in *C. borealis*) and regularly arranged contextual hyphae (interwoven in the type species). Furthermore, phylogenetic inferences based on a combined dataset of ITS, nLSU-rDNA and EF1α regions revealed that our specimens and specimens of *C. borealis* formed two distinct lineages. We therefore concluded that our chinese collections represent a new species, described below as *Climacocystis montana* sp. nov. Illustrated descriptions of the two *Climacocystis* species are provided.

Basidiomycota / Molecular phylogeny / Taxonomy / wood-rot fungi

INTRODUCTION

Climacocystis Kotl. & Pouzar is a monotypic genus typified by *Climacocystis borealis* (Fr.) Kotl. & Pouzar (Kotlába & Pouzar, 1958). Morphologically, it is characterized by an annual growth habit, pileate basidiocarps, a monomitic hyphal system with clamp connections, acute, thick-walled and ventricose cystidia, and thin-walled and broadly ellipsoid basidiospores which are negative in Melzer's reagent. It is widely distributed in the northern hemisphere (Gilbertson & Ryvarden, 1986; Ryvarden & Gilbertson, 1993; Núñez & Ryvarden, 2001; Dai, 2012).

Recent phylogenetic analysis shows the genus *Climacocystis* is placed in the residual polyporoid clade and stand closely to *Physisporinus* P. Karst., *Steccherinum* Gray and *Diplomitoporus* Domański and so on. They are known to produce a white-rot wood decay (Binder *et al.*, 2013; Miettinen *et al.*, 2011).

Eastern Himalayas is one of the hotspots for biodiversity. The local flora of seed plants is diverse. Many endemic species of woody plants occur locally and provide good substrates for wood-inhabiting fungi. Several new fungal taxa were recently described in these areas (Dai, 2011; Cui & Zhao, 2012; Zhou & Dai, 2012; Li & Cui, 2013a, b; Tian *et al.*, 2013; Zhao & Cui, 2013a, b; Zhao *et al.*, 2013; Chen & Cui, 2014; Chen *et al.*, 2014).

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During continuous surveys of wood-inhabiting fungi in eastern Himalayas, several specimens of *Climacocystis* were collected, and were found to deviate in several microscopic features from the type species, *C. borealis*, from which we conclude that they represent an undescribed species. In order to confirm the affinities of this new species, sequences of their internal transcribed spacer (ITS), nuclear large subunit (nLSU) and the elongation factors 1α (EF1α) regions were generated and compared with sequences of its related species.

MATERIAL AND METHODS

Morphological studies

The studied specimens were deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Microscopic examinations followed Dai *et al.* (2010). Sections were studied at a magnification up to 1000× using a Nikon E 80i microscope and phase contrast illumination (Nikon, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with cotton blue and Melzer's reagent. Spores were measured from sections cut from the tubes. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range and were given in parentheses. In the text the following abbreviations were used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), R = mean of L/W ratios, Q = variation in the L/W ratios between the specimens studied, n = number of spores measured from given number of specimens. Special color terms followed Petersen (1996).

Molecular procedures and phylogenetic analysis

The fungal specimens used in this study were listed in Table 1.

A Phire® Plant Direct PCR Kit (Finnzymes, Vantaa, Finland) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen was lysed in 30 µl dilution buffer for DNA extraction. After incubating 3 min at room temperature, 0.75 µl of the supernatant was used as template for a 30 µl PCR reaction. The ITS regions were amplified with the primers ITS4 and ITS5 (White *et al.*, 1990), LR0R and LR7 for nLSU regions, EF1-983F and EF1-1567R for EF1α regions (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS and EF1α was as follows: initial denaturation at 95°C for 3 min, followed by 34 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 34 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (Table 1).

Table 1. A list of species, specimens and GenBank accession number of sequences used in this study

Species	*Specimen no.*	*GenBank no.*		
		ITS	*nLSU*	*EF1-α*
Abortiporus biennis (Bull.) Singer	EL 65/03	JN649325	JN649325	JX109892
Antrodiella americana Ryvarden & Gilb.	KHL 11949	JN710509	JN710509	JN710711
A. semisupina (Berk. & M.A. Curtis) Ryvarden	KHL 11977	JX109842	JX109842	JX109896
Climacocystis borealis (Fr.) Kotl. & Pouzar	KHL 13318	JQ031126	JQ031126	JX109909
C. borealis	Dai 3703	KJ566626 [a]	KJ566636 [a]	KJ566643 [a]
C. borealis	Dai 4014	KJ566627 [a]	KJ566637 [a]	KJ566644 [a]
C. borealis	Dai 13208	KJ566635 [a]	KJ566642 [a]	–
C. borealis	Dai 11798	KJ566632 [a]	KJ566641 [a]	–
C. montana B.K. Cui & J. Song	Cui 9603	KJ566628 [a]	KJ566638 [a]	KJ566645 [a]
C. montana	Cui 9607	KJ566629 [a]	KJ566639 [a]	KJ566646 [a]
C. montana	Cui 9610	KJ566630 [a]	–	KJ566647 [a]
C. montana	Cui 9612	KJ566631 [a]	KJ566640 [a]	KJ566648 [a]
C. montana	Cui 10603	KJ566634 [a]	–	KJ566649 [a]
Hypochnicium lyndoniae (D.A. Reid) Hjortstam	NL 041031	JX124704	JX124704	JX109905
H. subrigescens Boidin	KHL 11968	JQ031128	JQ031128	JX109906
H. subrigescens	5285	JN710546	JN710546	–
Junghuhnia autumnale Spirin, Zmitr. & Malysheva	Spring 2957	JN710549	JN710549	JN710716
J. collabens (Fr.) Ryvarden	KHL 11848	JN710552	JN710552	JN710717
J. japonica Núñez & Ryvarden	Nuñez 1065	JN710556	JN710556	JN710718
J. luteoalba (P. Karst.) Ryvarden	KHL 13238b	JN710558	JN710558	JN710719
J. micropora Spirin	Spirin 2652	JN710559	JN710559	JN710720
J. nitida (Pers.) Ryvarden	KHL 11903	JN710560	JN710560	JN710721
J. pseudozilingiana (Parmasto) Ryvarden	Matti Kulju 1004	JN710561	JN710561	JN710722
Nigroporus vinosus (Berk.) Murrill	Miettinen 13139	JN710575	JN710575	–
N. vinosus	Seitzman 2008-100	JN710576	JN710576	JN710728
N. vinosus	BHS2008-100	JX109857	JX109857	JX109914
Oligoporus rennyi (Berk. & Broome) Donk	TN 7389	JX109849	JX109849	JX109903
Physisporinus sanguinolentus (Alb. & Schwein.) Pilát	KHL 11913	JX109843	JX109843	JX109897
P. vitreus (Pers.) P. Karst	KHL 11959	JQ031129	JQ031129	–
Podoscypha multizonata (Berk. & Broome) Pat.	KHM 12.X.1975	JN710581	JN710581	–
P. venustula (Speg.) D.A. Reid	LR 40821	JX109851	JX109851	JX109910
Spongipellis pachyodon (Pers.) Kotl. & Pouzar	AFTOL-ID 705	DQ249277	AY629322	DQ028599
Steccherinum fimbriatum (Pers.) J. Erikss	KHL 11905	JN710530	JN710530	–
S. ochraceum (Pers.) Gray	Ryberg s.n.	JN710589	JN710589	JN710730
S. ochraceum	KHL 11902	JQ031130	JQ031130	JX109893
S. ochraceum	3144	JN710590	JN710590	–
S. sp.	Miettinen 13705	JN710592	JN710592	JN710731
S. sp.	Miettinen 14391	JN710594	JN710594	JN710732
S. tenue Burds. & Nakasone	KHL 12316	JN710598	JN710598	JN710733
Xanthoporus syringae (Parmasto) Audet	AFTOL-ID 774	AY789078	AY684166	DQ059049
X. syringae	Laitakari 15.6.1999	JN710606	JN710606	–

[a] Sequences newly generated in this study

Sequences generated for this study were aligned with additional sequences downloaded from GenBank (Table 1) using BioEdit (Hall, 1999) and ClustalX (Thompson *et al.*, 1997). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. Sequence alignment was deposited at TreeBASE (http://purl.org/phylo/treebase; submission ID 15944).

Phylogenetic analysis followed Li & Cui (2013b). Maximum parsimony analysis was applied to the combined dataset of ITS, nLSU and EF1α sequences. Sequences of *Oligoporus rennyi* (Berk. & Broome) Donk obtained from GenBank were used as outgroup to root trees. The tree construction procedure was performed in PAUP* version 4.0b10 (Swofford, 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein, 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated.

MrMODELTEST2.3 (Posada & Crandall, 1998; Nylander, 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck, 2003). Four Markov chains were run for 2 runs from random starting trees for 2 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP) were considered as significantly supported, respectively.

RESULTS

The combined dataset include sequences from 41 fungal specimens representing 26 species. The dataset has an aligned length of 2,703 characters, of which 1,449 characters are constant, 322 are variable and parsimony-uninformative, and 932 are parsimony-informative. Maximum parsimony analysis yielded 5 equally parsimonious trees (TL = 3981, CI = 0.518, RI = 0.687, RC = 0.356, HI = 0.482), and a strict consensus tree of these trees is shown in Fig. 1. The best estimated model for ITS+nLSU+EF1α and applied in the Bayesian analysis is GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in a topology similar to the MP analysis with an average standard deviation of split frequencies = 0.005597.

Five samples of *Climacocystis borealis* from China, Finland, Estonia and Switzerland clustered in a distinct lineage in the phylogenetic analysis. Five specimens of the new species collected from the Himalayas and adjacent areas

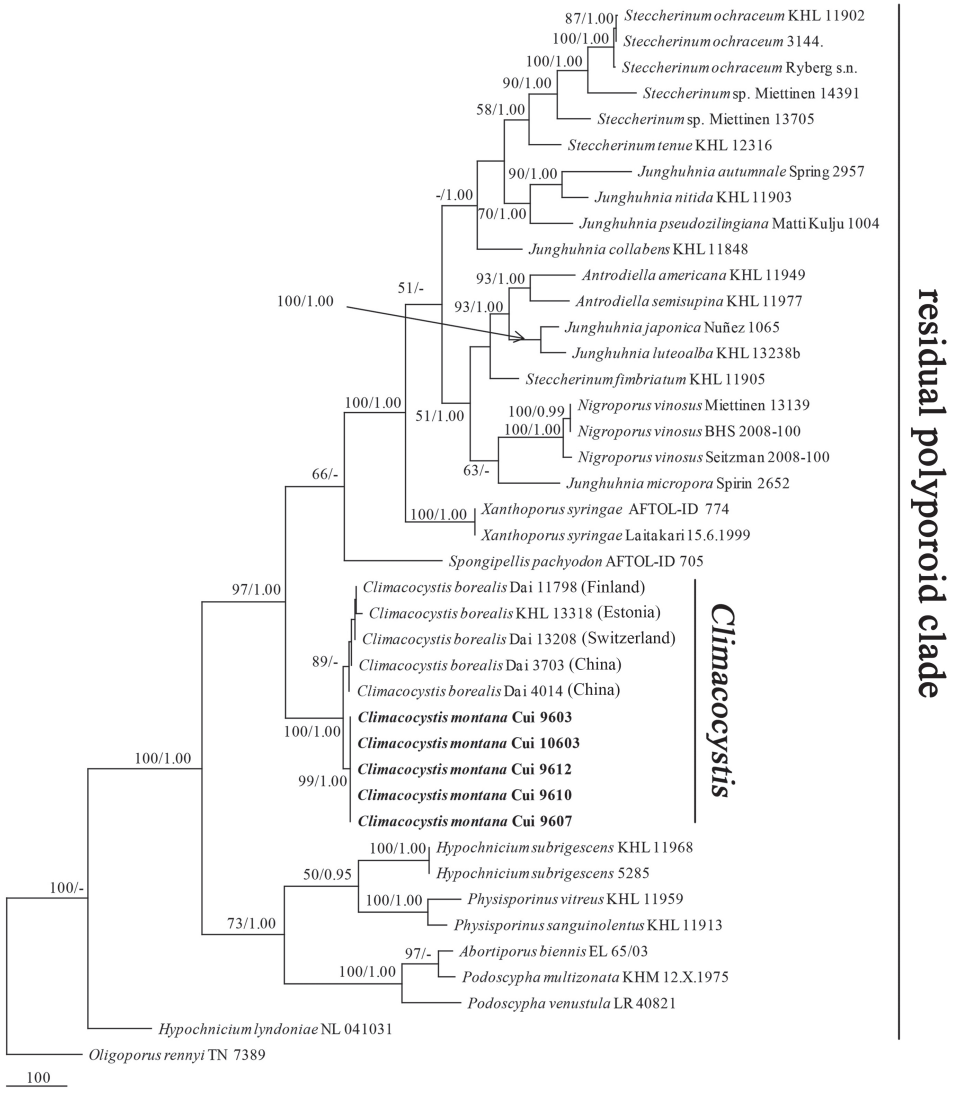

Fig. 1. Strict consensus tree illustrating the phylogeny of *Climacocystis* and related species generated by maximum parsimony based on ITS+nLSU+EF1α sequence data. Branches are labeled with parsimony bootstrap proportions (before the slash markers) high than 50% and bayesian posterior probabilities (after the slash markers) more than 0.95.

were grouped in a well-supported lineage (MP = 99%, BPP = 1.00). These two lineages formed the strongly supported *Climacocystis* group (MP = 100%, BPP = 1.00) and fell into the residual polyporoid clade (Fig. 1) according to Binder *et al.* (2013).

TAXONOMY

Climacocystis montana B.K. Cui & J. Song, **sp. nov.** **Figs 2a-b, 3**

MycoBank no.: MB 808338

HOLOTYPUS: **CHINA**, Xizang Autonomous Region (Tibet), Leiwuqi County, on fallen trunk of *Picea*, 22 Sep 2010, B.K. Cui, Cui 9603 (BJFC).

Etymology. *montana* (Lat.): referring to its locality in the high altitude of mountains.

Basidiocarps annual, pileate, sessile to laterally substipitate, usually imbricate, soft and watery when fresh, hard corky and light in weight when dry. **Pileus** applanate, fan-shaped to dimidiate, projecting up to 7 cm long, 14 cm wide and 2 cm thick at base. **Pileal surface** white to pale cream when fresh, becoming cream to yellowish-brown upon drying, often radially furrowed, azonate, tomentose to hirsute when fresh, becoming glabrous or tufted with short stiff hairs upon drying. **Pore surface** white to cream when fresh, becoming cream to clay-buff when dry; **pores** thin-walled angular, in part more irregular and split, 1-3 per mm; **dissepiments** thin, entire to lacerate. **Context** white to clay-buff, up to 1.3 cm thick. **Tubes** cream to clay-buff, crumbly, up to 7 mm long. **Type of rot.** White rot.

Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH. **Contextual** generative hyphae hyaline,

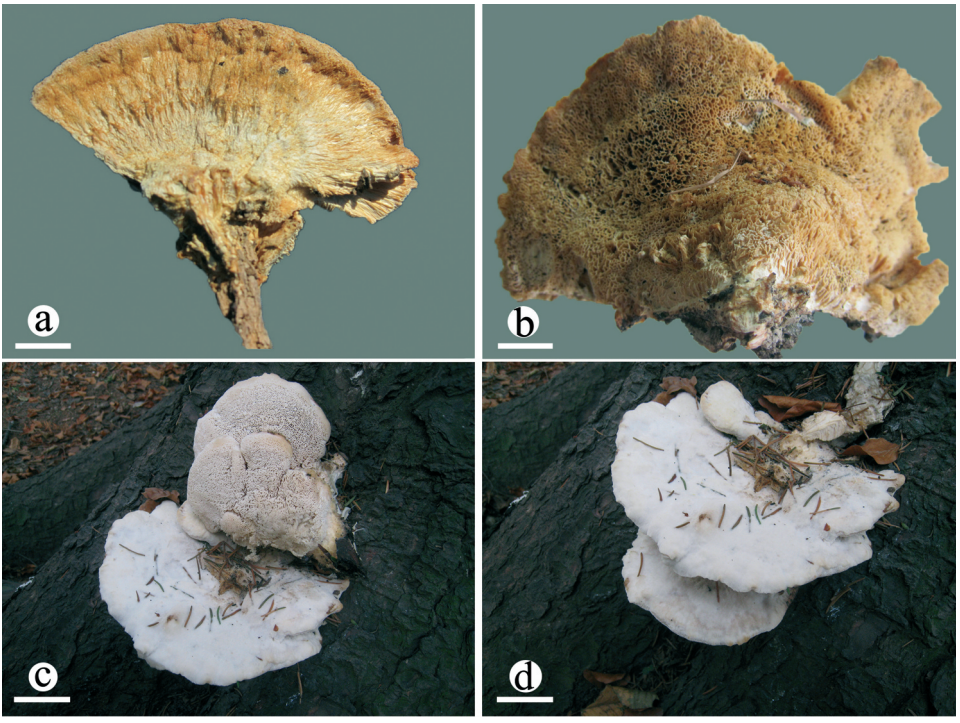

Fig. 2. Basidiomata of *Climacocystis* species. **a-b.** *C. montana*, **c-d.** *C. borealis*. Scale bars a-b = 1 cm, c = 2.5 cm, d = 1.5 cm.

thin- to mostly thick-walled, moderately branched, regularly arranged, 5-8 μm in diameter. **Hymenophoral trama** generative hyphae hyaline, thin- to thick-walled, moderately branched, interwoven, 3-4 μm in diameter. **Cystidia** numerous, ventricose, hyaline, smooth, without crystals, thin-walled at the base, becoming distinctly thick-walled towards the apex, 35-60 × 8-12 μm; **basidia** clavate, bearing four sterigmata and a basal clamp connection, 20-27 × 6-7 μm; **basidioles** dominant, similar in shape to basidia, but smaller. **Basidiospores** ellipsoid to subcilindrical, hyaline, thin-walled, smooth, IKI–, CB–, (5.5-)6-8.8(-10) × 3-4.2 μm, L = 7.19 μm, W = 3.85 μm, Q = 1.85-1.89 (n = 90/3), R = 1.87.

Additional specimens examined: **CHINA**. Xizang Autonomous Region (Tibet), Leiwuqi County, on fallen trunk of *Picea*, 22 Sep 2010, B.K. Cui, Cui 9610 (BJFC) & Cui 9612 (BJFC) & Cui 9607 (BJFC); Sichuan Province, Jiuzhaigou County, Jiuzhaigou Nature Resrve, on fallen trunk of *Picea*, 11 Oct 2012, B.K. Cui, Cui 10603 (BJFC).

***Climacocystis borealis* (Fr.) Kotl. & Pouzar,** Ceská Mykologie 12:96, 1958
Figs 2c-d, 4

– *Polyporus borealis* Fr., Systema Mycologicum 1: 366, 1821.

Basidiocarps annual, pileate, sessile to laterally substipitate, usually imbricate, soft and watery when fresh, hard corky and light in weight when dry. **Pileus** applanate, fan-shaped to dimidiate, projecting up to 11 cm long, 12 cm wide and 3 cm thick at base. **Pileal surface** white to cream when fresh, becoming buff yellow to orange-brown when dry, often radially furrowed, azonate, tomentose to hirsute when fresh, becoming glabrous or tufted with short stiff hairs upon drying; **margin** acute. **Pore surface** white to cream when fresh, becoming buff-yellow to orange-brown when dry; **pores** thin-walled and angular, in part more irregular and split, 1-3 per mm; **dissepiments** thin, lacerate. **Context** cream to buff, up to 1.8 cm thick. **Tubes** white to cream when fresh, becoming buff yellow to orange-brown when dry, up to 1.2 cm long. **Type of rot.** White rot.

Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH. **Contextual** generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, interwoven, 3-7 μm in diameter. **Hymenophoral trama** constitude by hyaline generative hyphae, mostly thin-walled, rarely thick-walled, moderately branched, interwoven, 2.5-4 μm in diameter. **Cystidia** numerous, ventricose, hyaline, smooth or apically encrusted, swelling in the middle part and tapering to the tip, thin-walled in the lower part, becoming distinctly thick-walled and slender towards the apex, 30-50 × 8-12 μm; **basidia** clavate, bearing four sterigmata and a basal clamp connection, 25-30 × 6-8 μm; **basidioles** dominant, in shape similar to basidia, but slightly smaller. **Basidiospores** ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, 5-6.8(-7) × (3-)3.2-4 μm, L = 5.95 μm, W = 3.63 μm, Q = 1.6-1.67 (n = 60/2), R = 1.64.

Specimens examined: **CHINA**. Heilongjiang Province, Yichun, Wuying County, Fenglin Nature Reserve, on stump of *Pinus*, 8 Sep 2002, Y.C. Dai, Dai 3703 (BJFC); Inner Mongolia Autonomous Region, Tongliao, Ganqika County, Daqinggou Park, on living tree of *Picea*, 24 Sep 2002, Y.C. Dai, Dai 4014 (BJFC). **FINLAND**. Helsinki, Vantaa, Tamisto Nature Resrve, on fallen trunk of *Picea*, 22 Sep 2010, Y.C. Dai, Dai 11798 (BJFC); on stump of *Picea*,15 Nov 2011, Y.C. Dai, Dai 12681 (BJFC). **SWITZERLAND**. Geneva, on living tree of *Picea*, 25 Nov 2012, Y.C. Dai, Dai 13208 (BJFC).

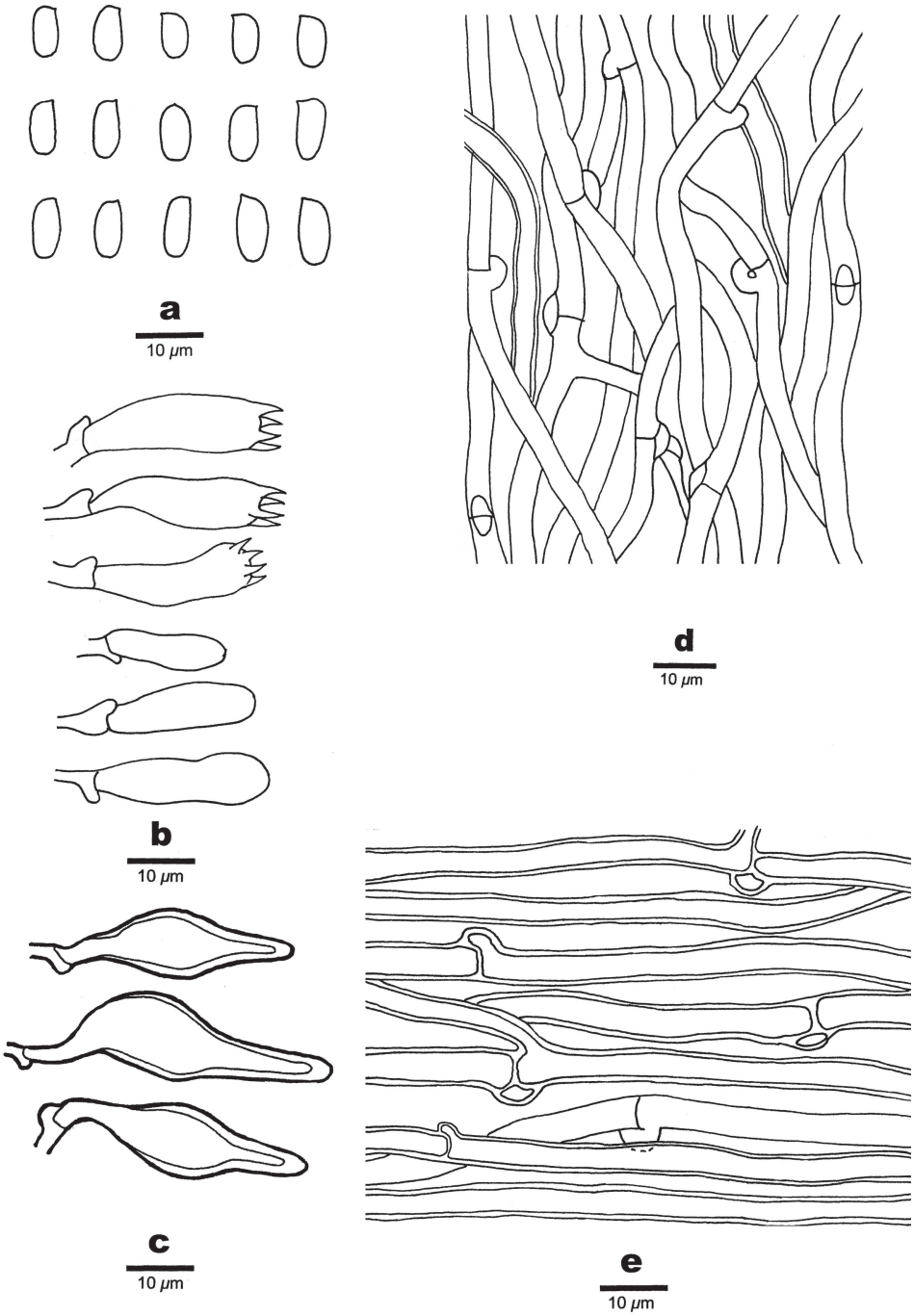

Fig. 3. Microscopic structures of *Climacocystis montana* (drawn from the holotype). **a.** Basidiospores. **b.** Basidia and basidioles. **c.** Cystidia. **d.** Hyphae from trama. **e.** Hyphae from context. Scale bars a-e = 10 μm.

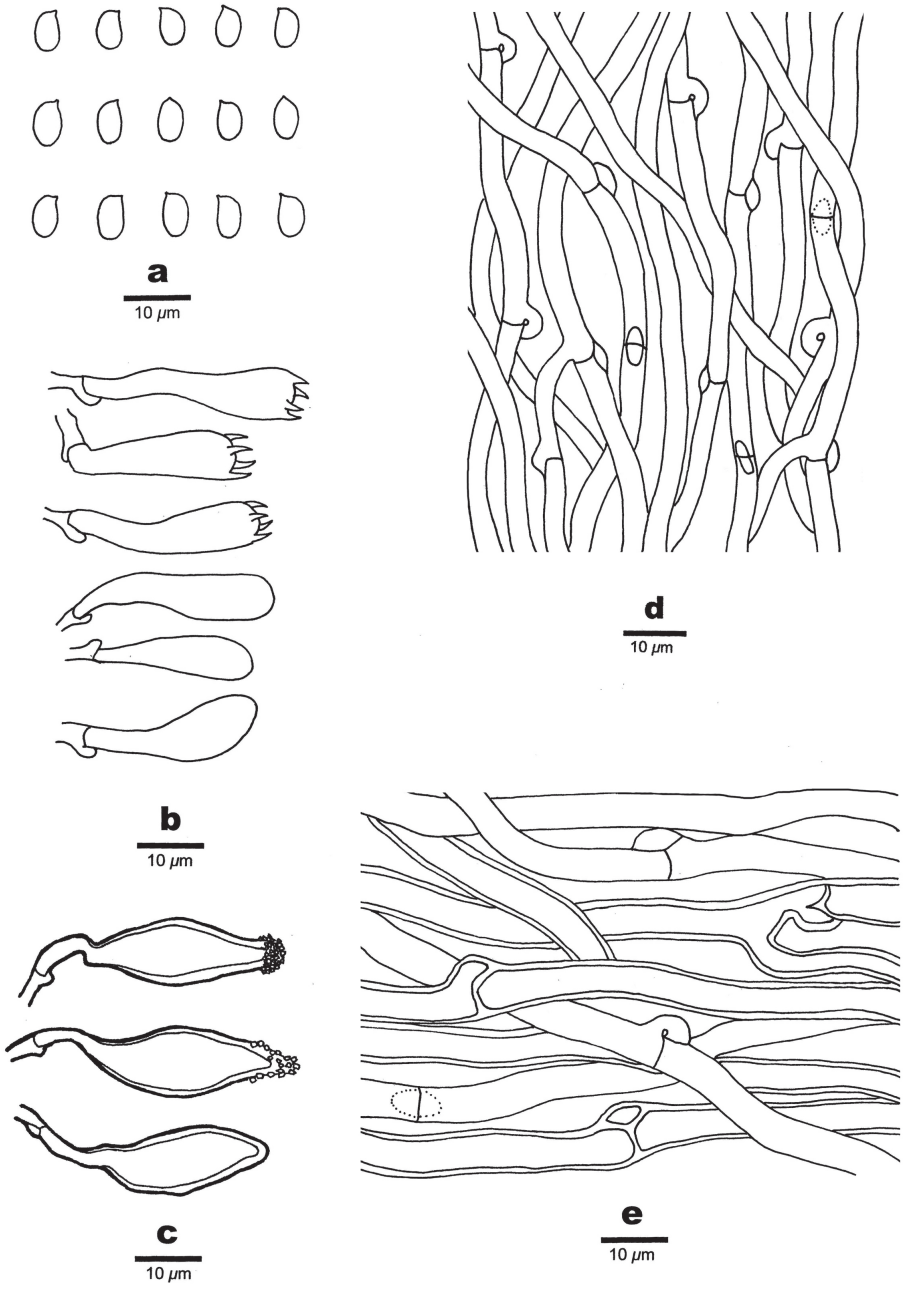

Fig. 4. Microscopic structures of *Climacocystis borealis* (drawn from Dai 3703). **a.** Basidiospores. **b.** Basidia and basidioles. **c.** Cystidia. **d.** Hyphae from trama. **e.** Hyphae from context. Scale bars a-e = 10 µm.

DISCUSSION

Climacocystis was a monotypic genus with *C. borealis*. In the present study, *C. montana* is described based on morphological differences and molecular phylogenetic analysis. Macrocopically, *C. montana* is similar to *C. borealis*, however both species differs microscopically. *Climacocystis montana* differs from *C. borealis* by smooth and ventricose cystidia, and ellipsoid to sub-silindrical basidiospores. Phylogenetic analysis inferred from the combined dataset of ITS, nLSU and EF1α sequences support that *C. montana* is a distinct species related to *C. borealis*.

Polypore diversity in China has been comprehensively reviewed by Dai (2012). *Climacocystis borealis* was reported as an uncommon species, though widely distributed in boreal areas of China, mainly found growing on *Abies*, *Picea* and *Pinus* (Dai, 2012). The species is more common in Europe and North America, where it is found growing mostly on *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* (Gilbertson & Ryvarden, 1986; Ryvarden & Gilbertson, 1993). *Climacocystis montana* has only been observed on *Picea* in the high altitude of mountains of southwestern China.

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