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Doi 10.5943/mycosphe/8/7/7

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A new species and a new combination of *Aleurodiscus* s.l. (Russulales, Basidiomycota)

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Dai LD, He SH. 2017 – A new species and a new combination of *Aleurodiscus* s.l. (Russulales, Basidiomycota). *Mycosphere* 8 (7), 908–916, Doi 10.5943/mycosphe/8/7/7

Abstract

Aleurodiscus ryvardeenii sp. nov. from southwestern China is described and illustrated. The species is characterized by possessing effused basidiocarps, simple-septate generative hyphae, moniliform gloeocystidia and verrucose basidiospores. *Aleurodiscus tsugae* on coniferous bark in East Asia is transferred to *Aleurocystidiellum*. Illustrated description of *Aleurocystidiellum tsugae* and a key to the three species of *Aleurocystidiellum* are given. The phylogeny of 29 species of *Aleurodiscus* s.l., *Stereum* and *Xylobolus* was inferred from ITS sequences.

Key words – *Aleurocystidiellum* – ITS – Stereaceae – taxonomy – wood-inhabiting fungi

Introduction

Aleurodiscus s.l. is an important group of wood-decaying fungi including a large number of species with very different morphological characters (Núñez & Ryvardeen 1997). Although several new species and new records have been discovered in subtropical and tropical China (Dai & He 2016, Dai et al. 2017a, b), the species diversity in these areas is still unclear. Recently, two specimens collected at Ailaoshan Nature Reserve, Yunnan Province, southwestern China is re-studied by morphological and molecular methods. They have totally effused basidiocarps, simple-septate generative hyphae, large moniliform gloeocystidia and strongly amyloid verrucose basidiospores, but are absent of acanthophyses. The effused yellow basidiocarps and large gloeocystidia resemble species of *Megalocystidium* Jülich which however differ in having clamped generative hyphae and smooth to minutely verrucose basidiospores. The species is described and illustrated as *Aleurodiscus ryvardeenii* new to science.

Aleurocystidiellum P.A. Lemke was erected by Lemke (1964) to accommodate *Aleurodiscus subcruentatus* (Berk. & M.A. Curtis) Burt which has discoid to stereoid basidiocarps, a dimitic hyphal system, encrusted skeletal cystidia and large verrucose basidiospores (Bernicchia & Gorjón 2010). Tellería (1990) transferred *Aleurodiscus disciformis* (DC.) Pat. to *Aleurocystidiellum*. Morphologically, *Aleurocystidiellum disciforme* (DC.) Tellería has moniliform gloeocystidia rather than skeletocystidia in *A. subcruentatum* (Berk. & M.A. Curtis) P.A. Lemke, but otherwise they are very similar. Meanwhile, the two-species formed a distinct lineage distant from *Aleurodiscus* s.s. in phylogenetic trees (Wu et al. 2001, Larsson & Larsson 2003, Binder et al. 2005). *Aleurodiscus tsugae* was originally described from Japan on bark of *Tsuga*, and then found on *Pinus* in Japan, the Russian Far East and northeastern China (Maekawa 1993, Núñez & Ryvardeen 1997, Wu 2008). Careful

morphological and molecular studies of the Chinese specimens of *Aleurodiscus tsugae* show that it belongs *Aleurocystidiellum*, and thus a new combination is proposed.

Materials & Methods

Morphological studies – Voucher specimens are deposited at the herbarium of Beijing Forestry University (BJFC), Beijing, China. Samples for microscopic examination were mounted in Melzer's reagent or 1% phloxine, and the procedures followed Chen et al. (2016). The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from number of specimens (b). Color names and codes are from Kornerup & Wanscher (1978).

DNA extraction and sequencing – A CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd) was employed for DNA extraction and PCR amplification from dried specimens. The ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990) using the following procedure: initial denaturation at 95 °C for 4 min, followed by 34 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and final extension at 72 °C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and the sequences were deposited in GenBank (Table 1).

Phylogenetic analyses – The molecular phylogeny was inferred from a dataset of 31 ITS sequences representing 24 *Aleurodiscus* s.l., 4 *Stereum* Hill ex Pers., one *Xylobolus* P. Karst and one outgroup species. Six sequences are newly generated, while others are mainly from Dai & He (2016) and Dai et al. (2017a, Table 1). *Heterobasidion parviporum* Niemelä & Korhonen was selected as outgroup (Dai & He 2016). The sequences were aligned using ClustalX v.1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit v.7.0.5.3 (Hall 1999), and then deposited at TreeBase (<http://treebase.org/treebase-web/home.html>, submission ID 21170).

Maximum parsimony analysis (MP) were performed using PAUP* 4.0b10 (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analyses were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each generated tree.

For Bayesian inference (BI), best models of evolution were obtained using MrModeltest v.2.2 (Nylander 2004), and posterior probabilities (BPP) were obtained by Markov Chain Monte Carlo sampling in MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run for five million generations for the dataset, and trees were sampled every 100th generation. The first quarter of the trees, which represented the burn-in phase of the analyses, were discarded, and the remaining trees were used to calculate posterior probabilities in the majority rule consensus tree.

Phylogenetic Results

The ITS dataset had an aligned length of 649 characters, of which 328 are parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 1456, CI = 0.530, RI = 0.630, RC = 0.334, HI = 0.470). The best-fit evolution model for BI was “GTR+I+G”. BI resulted in a topology similar to that of MP analysis with an average standard deviation of split frequencies = 0.006390. The strict consensus MP tree is shown in Fig. 1 with both BT values ($\geq 50\%$) and BPPs (≥ 0.95) labelled along the branches. In the tree, *Aleurodiscus ryvardeenii* forms a distinct lineage. *Aleurocystidiellum disciforme*, *A. subcruentatum* and *A. tsugae* form a strongly supported clade (BT = 100%, BPPs = 1.00).

Table 1 Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold

Taxa	Voucher	Locality	ITS
<i>Acanthobasidium bambusicola</i>	He 2357	China	KU559343
<i>Acanthobasidium weirii</i>	HHB 13132	Italy	KX306882
<i>Aleurocystidiellum disciforme</i>	He 3159	China	KU559340
<i>Aleurocystidiellum tsugae</i>	He 4167	China	KY706209
<i>Aleurocystidiellum tsugae</i>	He 4025	China	KY706211
<i>Aleurocystidiellum subcruentatum</i>	He 2886	China	KU559341
<i>Aleurodiscus amorphous</i>	Ghobad-Nejhad 2464	China	KU559342
<i>Aleurodiscus aurantius</i>	KC 565	UK	HQ441573
<i>Aleurodiscus botryosus</i>	He 2712	China	KX306877
<i>Aleurodiscus canadensis</i>	Wu 1207-90	China	KY706203
<i>Aleurodiscus cerussatus</i>	He 3007	China	KX306875
<i>Aleurodiscus dextrinoideocerussatus</i>	He 2820	China	KY706206
<i>Aleurodiscus effusus</i>	He 2261	China	KU559344
<i>Aleurodiscus gigasporus</i>	Wu 0108-15	China	KY706205
<i>Aleurodiscus grantii</i>	He 2895	China	KU559347
<i>Aleurodiscus ljubarskii</i>	He 2418	China	KU559349
<i>Aleurodiscus mesaverdensis</i>	FP 120155	USA	KU559359
<i>Aleurodiscus mirabilis</i>	He 3730	China	KX306878
<i>Aleurodiscus oakesii</i>	He 2243	USA	KU559352
<i>Aleurodiscus penicillatus</i>	HHB 14115	USA	KU559338
<i>Aleurodiscus ryvardeenii</i>	He 2648	China	KX306879
<i>Aleurodiscus tenuissimus</i>	He 3575	China	KX306880
<i>Aleurodiscus tropicus</i>	He 3833	China	KX553875
<i>Aleurodiscus wakefieldiae</i>	He 2580	China	KU559353
<i>Heterobasidion parviporum</i>	091605	China	KJ651503
<i>Neoaleurodiscus fujii</i>	He 2921	China	KU559357
<i>Stereum complicatum</i>	He 2234	USA	KU559368
<i>Stereum ostrea</i>	He 2067	USA	KU559366
<i>Stereum sanguinolentum</i>	He 2111	USA	KU559367
<i>Stereum spectabile</i>	275-597	Japan	AB509853
<i>Xylobous frustulatus</i>	He 2231	USA	KU881905

Taxonomy

Aleurodiscus ryvardeenii S.H. He & Y.C. Dai, **sp. nov.**

(Figs 2a, 3)

Mycobank number: MB 821576; Facesoffungi number: FoF 03397

Type – China, Yunnan Province, Jingdong County, Ailaoshan Nature Reserve, alt. 2500 m, on dead angiosperm branch, 25 August 2015, He 2648 (BJFC 022006, holotype).

Etymology – Named in honor of Prof. Leif Ryvardeen (University of Oslo, Norway), the author of the monograph of *Aleurodiscus* s.l.

Fruiting body – Basidiocarps annual, resupinate, effused, closely adnate, inseparable, membranous to coriaceous, soft, up to 16 cm long, 2 cm wide, 0.2 mm thick. Hymenophore smooth, pale orange (6A3), greyish orange [6B(3–6)] to brownish orange [6C(3–8)], not cracked; margin determinate, indistinct, concolorous or slightly darker than hymenophore surface.

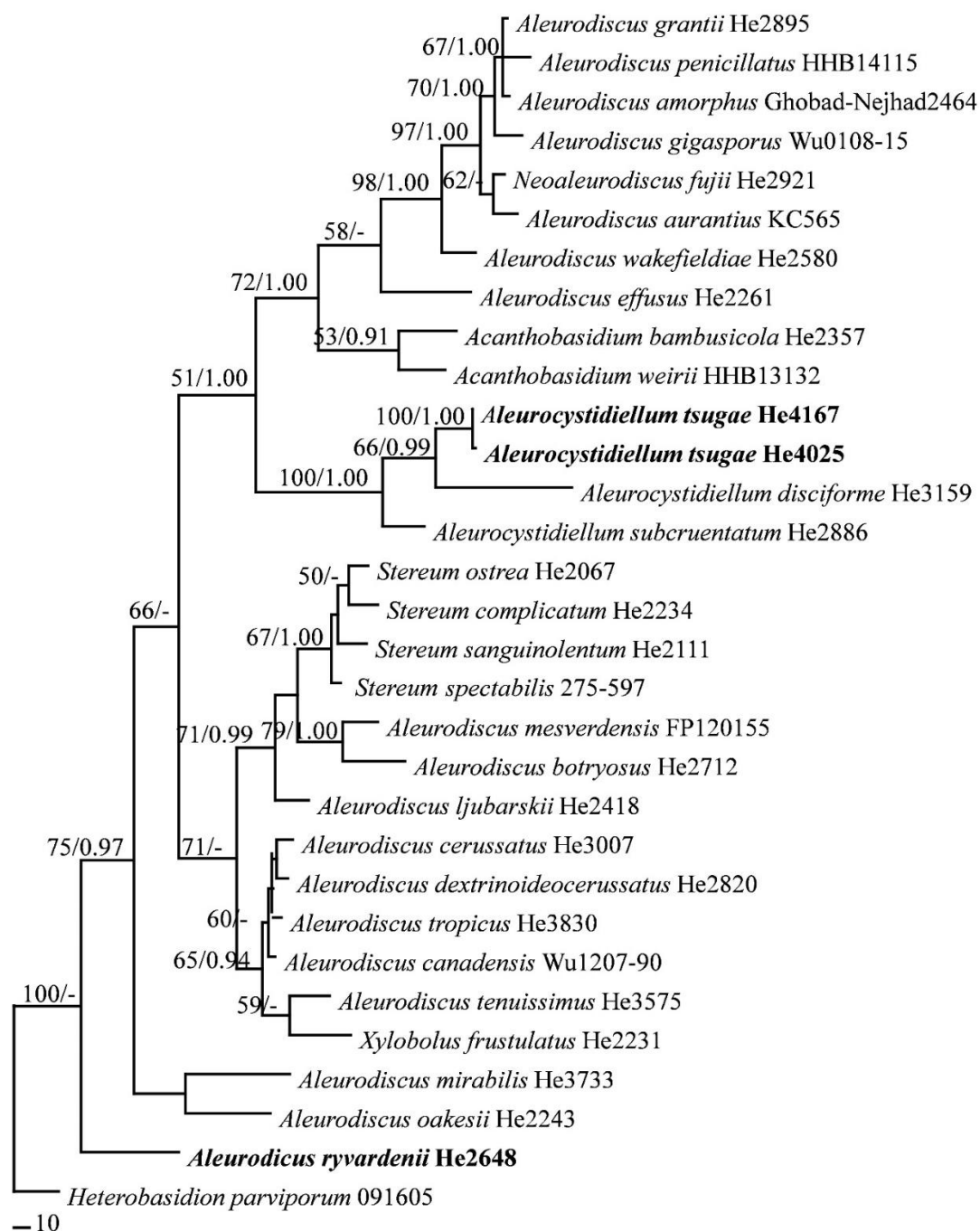


Figure 1 – Maximum parsimony strict consensus tree illustrating the phylogeny of species of *Aleurodiscus* s.l. and related genera based on ITS sequences. Branches are labelled with parsimony bootstrap values (before slash) $\geq 50\%$ and Bayesian posterior probabilities (after slash) ≥ 0.95 .

Microscopic structure – Hyphal system monomitic. Subiculum indistinct to almost lacking. Generative hyphae abundant, simple-septate, thin- to slightly thick-walled, frequently septate and branched, usually constricted at the septa, mostly vertically arranged, more or less agglutinated, intermingled with gloeocystidia, 2.5–5.5 μm in diam. Gloeocystidia abundant, moniliform with several constrictions, tapering toward the apex, sinuous, easily broken, slightly thick-walled, 85–110 (–160) \times 11–15 (–18) μm . Simple hyphidia present, thin-walled, hyaline. Basidia subclavate to subcylindrical, hyaline, slightly thick-walled, with 4 sterigmata and a basal simple septum, 50–65 \times 15–22 μm ; basidioles similar to basidia but smaller. Basidiospores broadly ellipsoid to subglobose, hyaline, thick-walled, verrucose, bearing an apiculus, strongly amyloid, 12–17 (–18) \times 10–15 (–16.5) μm , L = 14.5 μm , W = 13.2 μm , Q = 1.1 (n = 60/2).

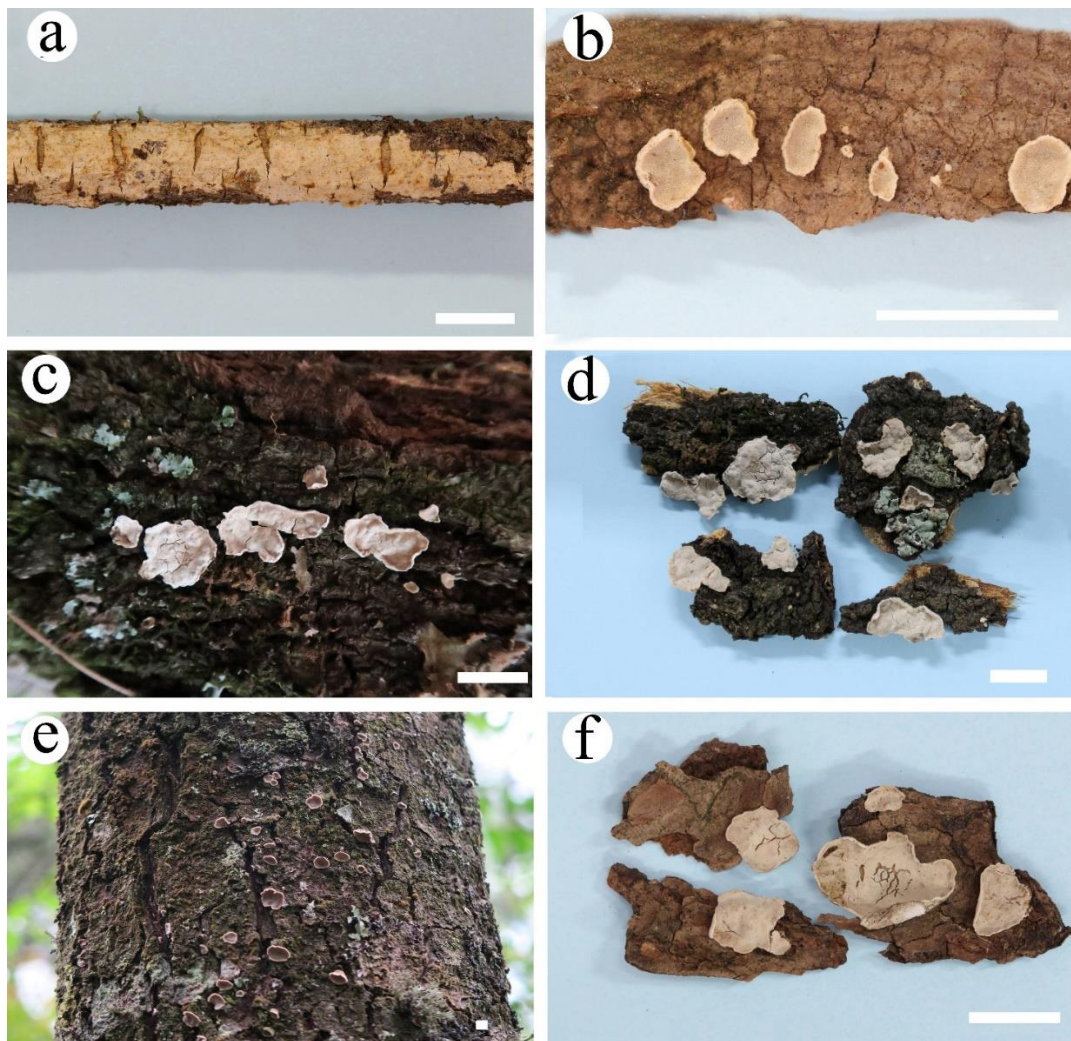


Figure 2 – Basidiocarps of *Aleurodiscus* s.l. species. a: *Aleurodiscus ryvardeenii* (holotype, He 2648); b: *Aleurocystidiellum subcruentatum* (He 2886); c–d: *Aleurocystidiellum disciforme* (He 3159); e–f: *Aleurocystidiellum tsugae* (e: He 3734; f: He 2326). Scale bars = 1 cm.

Additional specimen examined – China, Yunnan Province, Jingdong County, Ailaoshan Nature Reserve, alt. 2500 m, on dead angiosperm branch, 25 August 2015, He 2652 (BJFC 021093, paratype).

Remarks: Although *Aleurodiscus ryvardeenii* forms a distinct lineage distant from *A. amorphus* (Pers.) J. Schröt. (generic type) in the phylogenetic tree (Fig. 1), we place it in *Aleurodiscus* sensu Núñez & Ryvardeen (1997) according to morphological characters. *Aleurodiscus ryvardeenii* is similar to *A. cremicolor* Hjortstam & Ryvardeen by sharing corticioid basidiocarps, simple-septate generative hyphae, numerous gloeocystidia and verrucose basidiospores. However, *A. cremicolor* has smaller gloeocystidia ($40\text{--}60 \times 7\text{--}10 \mu\text{m}$) and narrower basidiospores ($12\text{--}14 \times 6\text{--}8 \mu\text{m}$, Núñez & Ryvardeen 1997). *Aleurodiscus lacunae-euimortui* J.A. Simpson & Grgur. reported from Australia also has simple-septate generative hyphae and large gloeocystidia, but differs from *A. ryvardeenii* by having numerous dendrohyphidia and larger basidiospores ($17\text{--}31.8 \times 15\text{--}21 \mu\text{m}$, Simpson & Grgurinovic 2003).

Aleurocystidiellum tsugae (Yasuda in Lloyd) S.H. He & Y.C. Dai, **comb. nov.** (Figs 2e-f, 4)

Mycobank number: MB 821577; Facesoffungi number: FoF 03398

Basionym: *Aleurodiscus tsugae* Yasuda in Lloyd, Mycological Writings 6 (65): 1066, 1920.

Synonym: *Acanthophysium tsugae* (Yasuda) Parmasto, Eesti NSV Teaduste Akadeemia Toimetised 14 (2): 220, 1965.

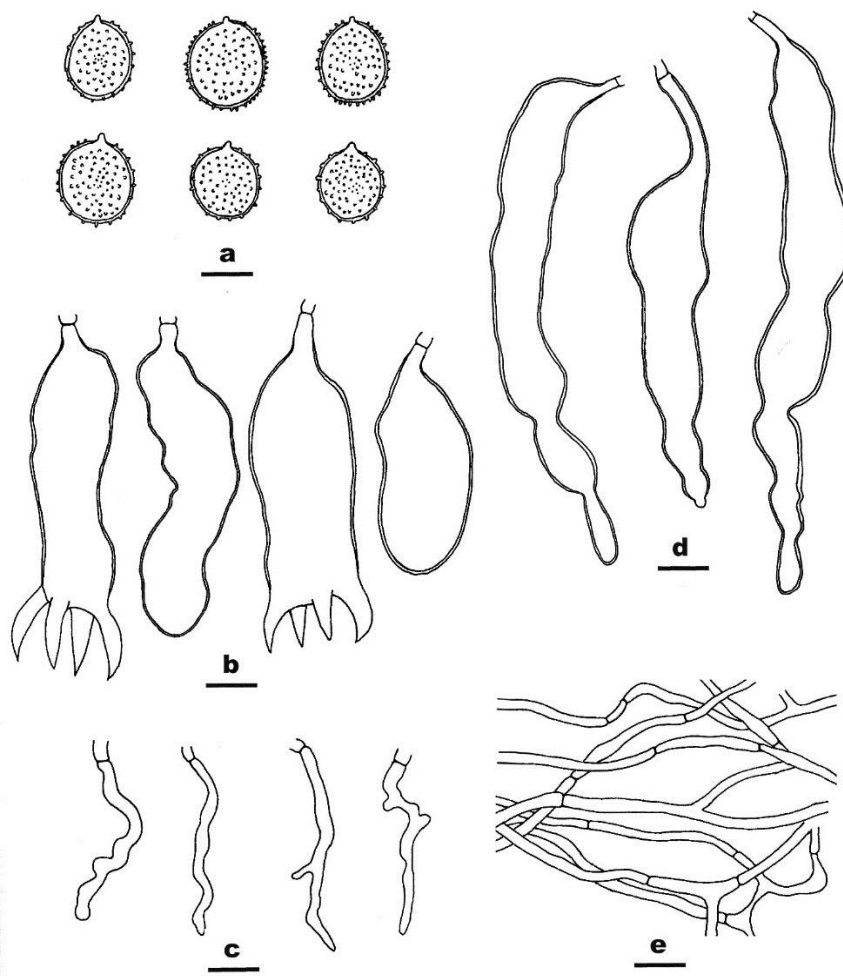


Figure 3 – Microscopic structures of *Aleurodiscus rywardenii* (holotype, He 2648). a: Basidiospores; b Basidia and basidioles; c: Hyphidia; d: Gloeocystidia; e: Generative hyphae. Scale bars = 10 μ m.

Fruiting body – Basidiocarps discoid, effused-reflexed to subpileate, easily detached from substrate, woody hard, effused parts up to 2.5 cm long, 1 cm wide, 0.1 cm thick, reflexed parts projecting up to 0.5 cm. Abhymenial surface orange grey (6B2), grey [6(B–F)1] to brownish grey (6F2); margin indistinct, sharp, curved inward, concolorous or slightly lighter than abhymenial surface. Hymenophore smooth, orange grey (6B2) to brownish grey [6(C–D)2], not cracked; margin thinning out, narrow, indistinct, slightly lighter than hymenophore surface.

Microscopic structure – Hyphal system monomitic. Subiculum thickening, generative hyphae in this layer thin- to thick-walled, hyaline, clamped, moderately branched and septate, sometimes distinctly thick-walled, very rarely branched and septate resembling skeletal hyphae, more or less regularly arranged, 2–6 μ m in diam. Subhymenium thickening, composed of old hymenial elements, generative hyphae in this layer thin- to thick-walled, vertically arranged, frequently branched and septate, more or less agglutinated. Acanthophyses absent. Gloeocystidia in hymenium numerous, moniliform, slightly thick-walled, 80–150 \times 10–13 μ m. Dendrohyphidia present, thin-walled, scattered. Basidia subclavate, slightly sinuous, hyaline, slightly thick-walled, with 4 sterigmata and a basal clamp connection, 60–100 \times 11–15 μ m; basidioles similar to basidia but smaller. Basidiospores ellipsoid to broadly ellipsoid, hyaline, thick-walled, verrucose, bearing a distinct apiculus, strongly amyloid, 15–20 (–24) \times 10–16 μ m, L = 17.5 μ m, W = 12.5 μ m, Q = 1.4 (n = 30/1).

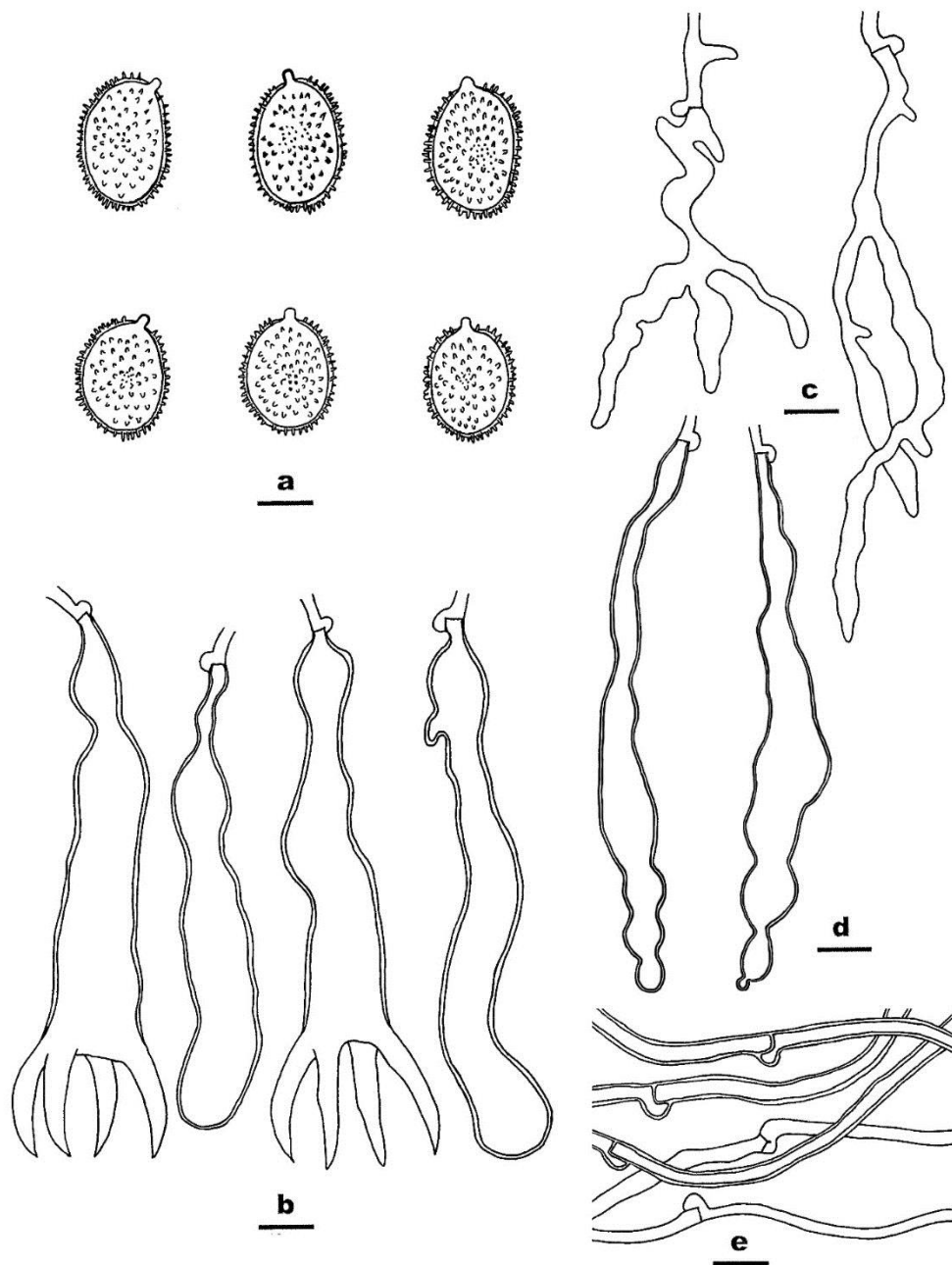


Figure 4 – Microscopic structures of *Aleurocystidiellum tsugae* (He 4024). a: Basidiospores; b: Basidia and basidioles; c: Dendrohyphidia; d: Gloeocystidia; e: Generative hyphae. Scale bars = 10 μ m.

Specimens examined – *Aleurocystidiellum tsugae*: China, Guizhou Province, Leishan County, Leigongshan Nature Reserve, on bark of dead *Pinus*, 12 June 2016, He 3734 (BJFC 022233) & He 3738 (BJFC 022237); Hunan Province, Liuyang County, Daweishan National Forest Park, on bark of dead *Pinus*, 10 July 2015, He 2326 (BJFC 020780); Jiangxi Province, Yifeng County, Guanshan Nature Reserve, 9 August 2016, He 4163 (BJFC 023605) & He 4167 (BJFC 023609); Jilin Province, Fusong County, Lushuihe, on fallen trunk of *Pinus*, 19 August 2007, Wu 0708-16; Yunnan Province, Kunming, Xishan Park, on bark of dead *Pinus*, 1 August 2016, He 4022 (BJFC 023461); He 4023 (BJFC 023462); He 4024 (BJFC 023463); He 4025 (BJFC 023464). *Aleurocystidiellum disciforme*: China, Yunnan Province, Mouding County, Huafohan Nature Reserve, on bark of living *Quercus*, 25 November 2015, He 3159 (BJFC 021554, Figs 2c–d). *Aleurocystidiellum subcruentatum*: China, Yunnan Province, Yulong County, Laojunshan Nature Reserve, alt. 3800 m, on bark of living *Abies*,

31 August 2015, He 2877 (BJFC 021311), He 2879 (BJFC 021312), He 2886 (BJFC 022012, Fig. 2b), He 2894 (BJFC 021325), He2898 (BJFC 021329), He 2910 (BJFC 021341), He 2911 (BJFC 022013), He 2914 (BJFC 021344).

Remarks: Wu (2008) recorded *Aleurocystidiellum tsugae* in northeastern China, and we found that it also has a wide distribution in southern China on bark of *Pinus*. The species is similar to *A. disciforme* by sharing discoid to effused-reflexed basidiocarps, moniliform gloecystida and verrucose basidiospores; however, the latter species has slightly smaller basidiospores (15–17 × 10–12 µm) and grows on angiosperms (Núñez & Ryvarden 1997). In our phylogenetic analyses, *A. tsugae*, *A. disciforme* and *A. subcruentatum* formed a fully supported clade (Fig. 1). Till now, all these three species of *Aleurocystidiellum* have been recorded in China (Dai 2011).

Key to *Aleurocystidiellum* species:

1. On angiosperms, mainly *Quercus*..... *A. disciforme*
1. On gymnosperms2
2. Encrusted skeletocystidia present*A. subcruentatum*
2. Moniliform gloecystidia present.....*A. tsugae*

Acknowledgements

Special thanks are due to Dr. Sheng-Hua Wu (National Museum of Natural Science, Taiwan) for specimen loans. We appreciate Prof. Yu-Cheng Dai (Beijing Forestry University, China) for his help in specimen collection and manuscript revision. The research was supported by the Fundamental Research Funds for the Central Universities (No. 2017PT09) and the National Natural Science Foundation of China (Nos. 31470144 & 31670013).

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