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Two new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) on bamboo from tropics

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ABSTRACT

Aleurodiscus tenuissimus and *A. tropicus*, on dead bamboo, are new species from tropical Asia. Both species have effused basidiocarps and simple-septate generative hyphae. *Aleurodiscus tenuissimus*, from southern China, lacks acanthophyses and possesses echinulate basidiospores, whereas *A. tropicus*, from southern China and Vietnam, has abundant acanthophyses and smooth basidiospores. Phylogenetic analyses of the nuc rDNA internal transcribed spacer region sequences (ITS1-5.8S-ITS2 = ITS) support the recognition of the taxa as distinct. The new species are described and illustrated, and a worldwide key to six species of *Aleurodiscus* on bamboo is provided.

1. Introduction

Aleurodiscus s.l. is a readily recognized group of wood-inhabiting fungi with cupulate, effused, or effused-reflexed basidiocarps, smooth or ornamented amyloid basidiospores, and sterile organs such as acanthophyses, gloeocystidia, and dendrohyphidia (Núñez and Ryvarden 1997; Bernicchia and Gorjón 2010). There are 25 species of *Aleurodiscus* s.l. reported from China (Dai et al. 2004; Dai 2011; Dai and He 2016), but species diversity and distribution of *Aleurodiscus* s.l. in tropical Asia are still poorly studied. In 2016, many corticioid or crust fungi were collected during field trips to Hainan Province, a tropical region in southern China. Some of the specimens were on bamboo which is widely distributed in eastern and southeastern Asia.

There are more than 1400 species of bamboo (Poaceae, Bambusoideae) worldwide (Kelchner and Bamboo Phylogeny

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Group 2013). Woody bamboo species support a number of wood-decay fungi reported by Hino and Katumoto (1961), Boidin et al. (1986), Hyde et al. (2002), Coelho et al. (2006, 2009), Cui et al. (2007, 2011), Choeyklin et al. (2009), Zhou and Jia (2010), Dai et al. (2011) and Zhao et al. (2014). Morphological and molecular studies of corticioid specimens from Hainan Province on bamboo revealed two new species of *Aleurodiscus* s.l. — *Aleurodiscus tenuissimus* and *A. tropicus*. The new taxa are described and illustrated, and a worldwide identification key is provided for all 6 known species of *Aleurodiscus* s.l. on bamboo.

2. Materials and methods

2.1. Morphological studies

The studied specimens are deposited in the herbaria of Beijing Forestry University (BJFC) and National Museum of Natural Science, Taiwan (TNM). Samples for microscopic examination were mounted in cotton blue, Melzer's reagent or 1% phloxine. The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) from number of specimens (b), CB− = acyanophilous, SA− = negative reaction in sulfobenzaldehyde. Color codes and names follow Kornerup and Wanscher (1978).

2.2. Molecular studies and phylogenetic analyses

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens. ITS regions were amplified with primers ITS4 and ITS5 (White et al. 1990). PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, 58 °C for 45 s, 72 °C for 1 min, and a final extension at 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).

Sequences generated in this study were aligned with related sequences obtained from GenBank (Table 1) using ClustalX v.1.83 (Chenna et al. 2003), and manually adjusted in BioEdit v.7.0.5.3 (Hall 1999). *Heterobasidion parviporum* Niemelä & Korhonen was selected as the outgroup taxon (Wu et al. 2001; Dai and He 2016). Sequence alignments were deposited at TreeBase (submission ID: 19573). Trees were figured in TreeView v.1.6.6 (Page 1996).

Maximum parsimony analyses (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 (BT) 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 indices (CI), retention indices (RI), rescaled consistency indices (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 determined by Markov Chain Monte Carlo sampling in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). Four simultaneous Markov chains were run for one million generations for the ITS dataset, and trees were sampled every 100th generation. The first 25% of the trees, which represented the burn-in phase of the analyses, were discarded, and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree.

3. Results

3.1. Molecular phylogeny

The ITS dataset contained 39 ITS sequences representing 24 in-group taxa and the out-group taxon, of which 14 sequences were newly generated (Table 1). This dataset had an aligned length of 646 characters of which 356 were parsimony informative. MP analysis yielded two equally parsimonious trees (TL = 1428, CI = 0.528, RI = 0.732, RC = 0.387, HI = 0.472). The best-fit evolution model for BI was "GTR+I+G". BI analyses resulted in a phylogenetic tree of similar topology to the MP tree with an average standard deviation of split frequencies = 0.008148. The strict consensus MP tree is shown in Fig. 1 with both BT values (≥50%) and BPPs (≥0.95) shown along the branches. In the tree, *Aleurodiscus tenuissimus* and *A. tropicus* each formed a distinct lineage. *Aleurodiscus tenuissimus* was sister to *Xylobolus frustulatus* (Pers.) P. Karst. (BT = 84%, BPPs < 0.95; Fig. 1), and *A. tropicus* sister to *A. cerussatus* (Bres.) Höhn. & Litsch. (BT = 85%, BPPs = 0.97; Fig. 1).

3.2. Taxonomy

Aleurodiscus tenuissimus L.D. Dai & S.H. He, sp. nov. Figs. 2A, 3. MycoBank no.: MB818335.

Diagnosis: The species is distinct by its effused, thin basidiocarps, simple-septate generative hyphae, lack of acanthophyses, thick-walled hyphidia, small basidia, echinulate basidiospores, and habit on dead bamboo in tropical areas.

Type: CHINA, Hainan Province, Lingshui County, Diaoluoshan Nature Reserve, on dead bamboo, 17 Mar 2016, S.H. He, He 3575 (holotype, BJFC 022075).

rRNA gene sequences ex-holotype: KX306880 (ITS), KX842529 (nLSU).

Etymology: "*tenuissimus*" (Lat.) refers to the thin basidiocarps.

Fruiting body: Basidiocarps annual, resupinate, effused, closely adnate, not separable, coriaceous, at first as small patches, later confluent up to 15 cm long, 40–55 μm thick. Hymenophore smooth, white (1A1) when fresh, becoming pale grey (1B1) or yellowish white (1B2) when dry, not cracked; margin abrupt, indistinct, concolorous with hymenophore surface.

Hyphal structure: Hyphal system monomitic. Subiculum absent, subhymenium thickening. Generative hyphae more or less interwoven, simple-septate, colorless, thin- to thick-walled, moderately branched, 1–3.5 μm diam.

Table 1 – Taxa with locality, substrate, and GenBank accession numbers for ITS sequences used in the phylogenetic analyses.

Taxa	Voucher	Locality	Substrate	ITS
Acanthobasidium bambusicola	He2357	China	Bamboo	KU559343
Acanthobasidium weirii	HHB13132	Italy	*Tsuga mertensiana*	KX306882[a]
Acanthobasidium weirii	HHB13499	Canada	*Tsuga heterophylla*	KX306883[a]
Aleurocystidiellum disciforme	He3159	China	*Quercus* sp.	KU559340
Aleurocystidiellum subcruentatum	He2886	China	*Abies* sp.	KU559341
Aleurocystidiellum subcruentatum	HHB17353	USA	*Picea glauca*	KU559360
Aleurodiscus amorphus	Ghobad-Nejhad2464	China	*Abies* sp.	KU559342
Aleurodiscus amorphus	HHB11320	USA	*Abies balsamea*	KU559362[a]
Aleurodiscus aurantius	KC565	United Kingdom	–	HQ441573
Aleurodiscus botryosus	He2712	China	Angiosperm	KX306877[a]
Aleurodiscus cerussatus	He2208	USA	Angiosperm	KX306874[a]
Aleurodiscus cerussatus	He3007	China	Angiosperm	KX306875[a]
Aleurodiscus effusus	He2261	China	Gymnosperm	KU559344
Aleurodiscus grantii	HHB14417	USA	*Abies procera*	KU559363
Aleurodiscus grantii	He2895	China	*Abies* sp.	KU559347
Aleurodiscus ljubarskii	He2418	China	*Quercus* sp.	KU559349
Aleurodiscus ljubarskii	TAA101173	Russia	–	KU559370[a]
Aleurodiscus mesaverdensis	FP120155	USA	–	KU559359
Aleurodiscus mirabilis	Dai13281	China	Angiosperm	KU559350
Aleurodiscus mirabilis	He3730	China	*Cinnamomeum*	KX306878[a]
Aleurodiscus oakesii	He2243	USA	*Quercus* sp.	KU559352
Aleurodiscus oakesii	HHB11890	USA	*Ostrya virginiana*	KU559365
Aleurodiscus penicillatus	HHB14115	USA	*Picea sitchensis*	KU559338[a]
Aleurodiscus tenuissimus	He3575	China	Bamboo	KX306880[a]
Aleurodiscus tenuissimus	Dai16124	China	Bamboo	KX306876[a]
Aleurodiscus tropicus	He3830	China	Bamboo	KX553875[a]
Aleurodiscus tropicus	He3834	China	Bamboo	KX553876[a]
Aleurodiscus tropicus	Wu9807-75	Vietnam	Bamboo	KX842530[a]
Aleurodiscus wakefieldiae	FP135654	France	*Castanea*	KU559369
Aleurodiscus wakefieldiae	He2580	China	Angiosperm	KU559353
Neoaleurodiscus fujii	He2921	China	*Rhododendron* sp.	KU559357
Neoaleurodiscus fujii	Wu0807-41	Japan	*Rhododendron* sp.	FJ799924
Stereum complicatum	He2234	USA	*Quercus* sp.	KU559368
Stereum ostrea	He2067	USA	*Quercus* sp.	KU559366
Stereum sanguinolentum	He2111	USA	*Pinus* sp.	KU559367
Xylobolus frustulatus	He2231	USA	*Quercus* sp.	KU881905
Xylobolus frustulatus	KUC20121102-07	South Korea	–	KJ668431
Xylobolus spectabilis	275–597	Japan	–	AB509853
Outgroup				
Heterobasidion parviporum	091605	China	–	KJ651503

[a] Sequences newly generated in this study.

Hymenium: Acanthophyses absent. Hyphidia abundant, simple or branched, colorless, thick-walled, sinuous, 23–48 × 2.5–4 μm. Gloeocystidia obclavate to submoniliform with an apical papilla, slightly sinuous, thick-walled, SA–, 34–61 × 6–12 μm. Basidia clavate to subcylindrical, usually with 1–2 constrictions, colorless, thick-walled, with 4 sterigmata, simple-septate at base, usually stalked, occasionally with a short lateral branch, 13–23 × 5.7–9.5 μm.

Spores: Basidiospores ellipsoid to subovoid, colorless, thick-walled, with a distinct apiculus, densely echinulate, strongly amyloid, CB–, (5–)6–8(–8.5) × (3–)4–5 μm, L = 6.8 μm, W = 4.2 μm, Q = 1.6–1.7 (n = 90/3).

Habitat and distribution: On dead bamboo stems, known from tropical China.

Type of rot: White rot.

Additional specimens examined: CHINA, Hainan Province, Lingshui County, Diaoluoshan Nature Reserve, on dead bamboo, 17 Mar 2016, S.H. He, He 3576 (BJFC 022076); 13 Nov 2015, Y.C. Dai, Dai 16124 (BJFC 020217).

Aleurodiscus tropicus L.D. Dai & S.H. He, sp. nov. Figs. 2B, 4. MycoBank no.: MB818336.

Diagnosis: The species is distinct by its effused basidiocarps, generative hyphae with simple septa, amyloid acanthophyses, abundant moniliform gloeocystidia, basidia with acanthophysoid appendages, smooth thin-walled basidiospores, and habit on dead bamboo in tropical areas.

Type: CHINA, Hainan Province, Qiongzhong County, Limushan Nature Reserve, on dead bamboo, 8 Jun 2016, S.H. He, He 3830 (holotype, BJFC 022332).

rRNA gene sequences ex-holotype: KX553875 (ITS); KX578720 (nLSU).

Etymology: "*tropicus*" (Lat.) refers to the distribution in tropical areas.

Fruiting body: Basidiocarps annual, resupinate, effused, closely adnate, not separable, soft coriaceous, first as small patches, later confluent up to 30 cm long, 150–220 μm thick. Hymenophore smooth, white (1A1), pale yellow (1A3) to

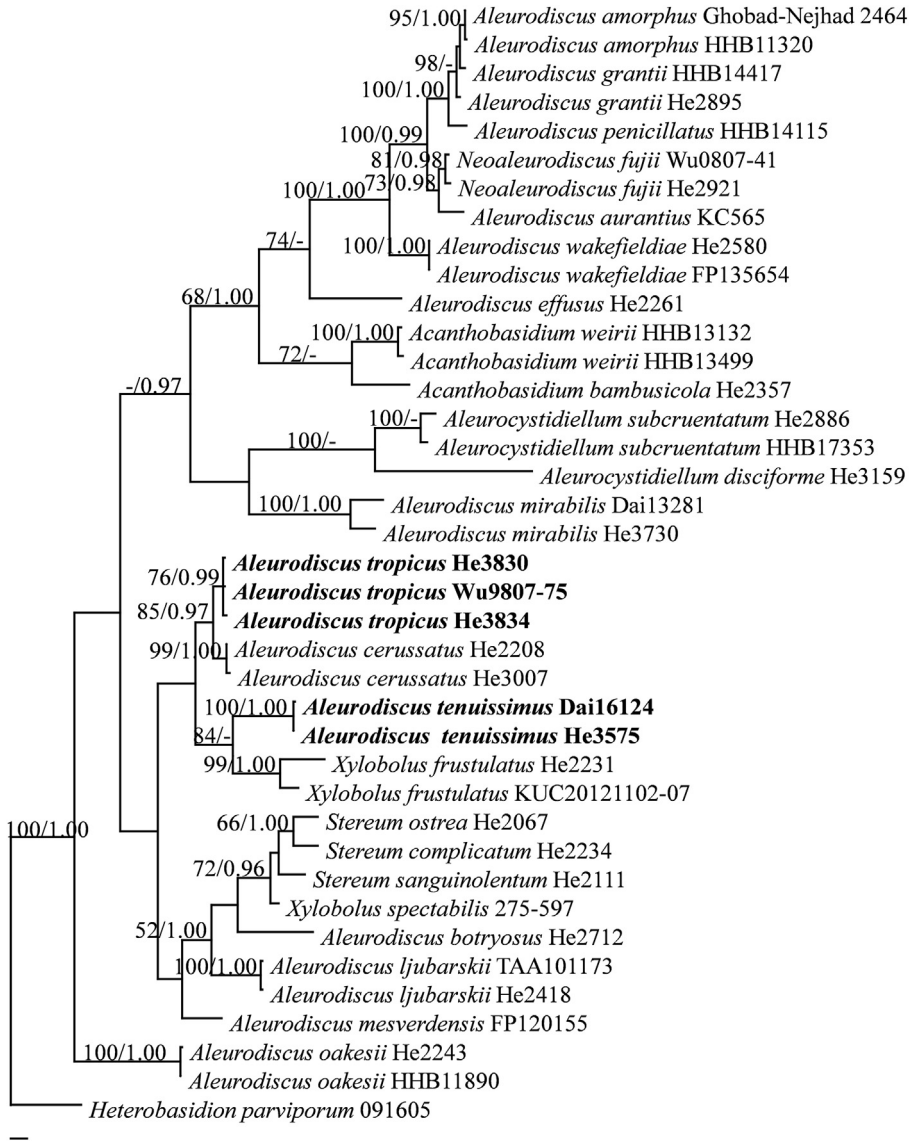

Fig. 1 – Maximum parsimony strict consensus tree illustrating the phylogeny of species of *Aleurodiscus* s.l. and related genera based on ITS sequences. Branches are labeled with parsimony bootstrap values (before slash) ≥50% and Bayesian posterior probabilities (after slash) ≥0.95. *Bar*: 10 steps.

greyish yellow [1B(3–7)], not cracked; margin abrupt, thinning out, slightly fimbriate, indistinct, concolorous with hymenophore surface.

Hyphal structure: Hyphal system monomitic. Subiculum indistinct to absent, usually embedded with empty gloeocystidia, generative hyphae next to substrate loosely interwoven, easily to discern, more or less parallel to the substrate, simple-septate, smooth, thick-walled, moderately branched, 2–3.5 μm diam. Subhymenium thickening, composed of generative hyphae like those of the subiculum and elements of post-mature hymenium.

Hymenium: Acanthophyses abundant, of two types: (1) subclavate to lobed with numerous, slightly amyloid protuberances at apex, 29–55 × 6–9 μm; (2) hyphoid with numerous slightly amyloid protuberances at apex, 30–50 × 2–3 μm. Gloeocystidia constricted, thick-walled, sinuous, containing numerous, small oil-drops, SA–, 53–85 × 12–17 μm. Basidia clavate to subcylindrical, colorless, thick-walled, with a lateral acanthophysoid appendage, with 4 sterigmata, simple-septate at base, 27–55 × 7–10 μm.

Spores: Basidiospores ellipsoid to oblong ellipsoid, smooth, colorless, thin-walled, amyloid, CB–, (8–)9–12(–13) × (4–)5–7.5(–8) μm, L = 10.6 μm, W = 6.2 μm, Q = 1.6–1.8 (n = 120/4).

Habitat and distribution: On dead bamboo stems, known from tropical Asia.

Type of rot: White rot.

Additional specimens examined: CHINA, Hainan Province, Qiongzhong County, Limushan Nature Reserve, on dead bamboo, 8 Jun 2016, S.H. He, He 3834 (BJFC 022336), He3841 (BJFC 022343), He 3845 (BJFC 022347), He 3850 (BJFC 022352) & He 3853 (BJFC 022355). Vietnam, Ba Vì National Park, alt. 650 m, on culm of bamboo, 04 Jul 1998, S.H. Wu & S.Z. Chen, Wu 9807-75 (TNM F0009060).

Fig. 2 – Basidiocarps of *Aleurodiscus* spp. A: *A. tenuissimus* (He 3576); B: *A. tropicus* (He 3841). *Bars*: A, B 1 cm.

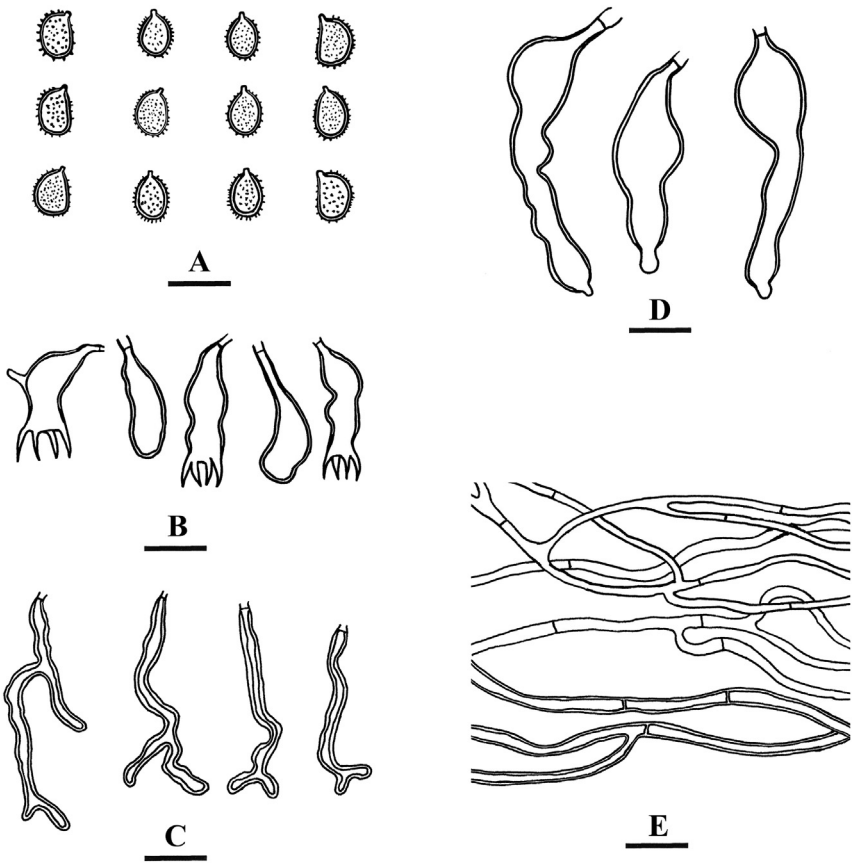

Fig. 3 – Microscopic structures of *Aleurodiscus tenuissimus* (He 3575, holotype). A: Basidiospores; B: Basidia and basidioles; C: Hyphidia; D: Gloeocystidia; E: Generative hyphae. *Bars*: A–E 10 μm.

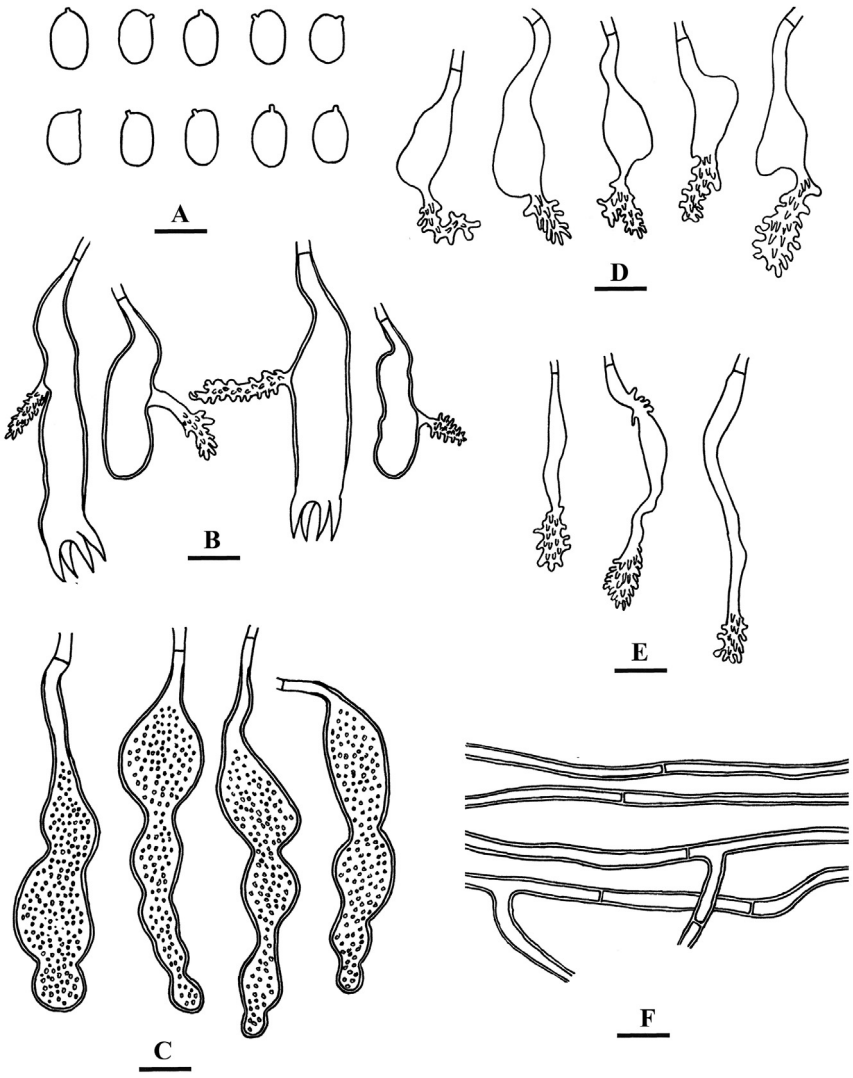

Fig. 4 – Microscopic structures of *Aleurodiscus tropicus* (He 3830, holotype). A: Basidiospores; B: Basidia and basidioles with acanthophysoid appendages; C: Gloeocystidia; D: Acanthophyses (clavate); E: Acanthophyses (hyphoid); F: Generative hyphae. *Bars*: A–F 10 μm.

4. Discussion

We use a broad, inclusive concept of *Aleurodiscus* as defined by Núñez and Ryvarden (1997) because the phylogenetic relationships of the species and genera in the Stereaceae Pilát are unresolved (Larsson and Larsson 2003; Miller et al. 2006; Larsson 2007). The generic limits of *Xylobolus* P. Karst., *Stereum* Hill ex Pers and some genera of *Aleurodiscus* s.l. have not been completely circumscribed (Wu et al. 2001; Larsson and Larsson 2003).

The new species *A. tenuissimus* and *A. tropicus* have similar effused basidiocarps, simple-septate generative hyphae, and substrate preference on dead bamboo stems. *Aleurodiscus tenuissimus*, however, can be easily distinguished from *A. tropicus* by the absence of acanthophyses and by its smaller, echinulate basidiospores.

Aleurodiscus tenuissimus and *A. phragmitis* (Boidin, Lanq., Cand., Gilles & Hugueney) Núñez & Ryvarden develop thin, effused basidiocarps, ornamented ellipsoid basidiospores, and occur on bamboo, but the latter has abundant acanthophyses, clamped generative hyphae, and large basidiospores (11–14 × 5.5–7 μm, Núñez and Ryvarden 1997). Although basidiospores of *Aleurodiscus weirii* Burt and *A. laurentianus* H.S. Jacks. & P.A. Lemke are similar to *A. tenuissimus*, they differ in having acanthophyses, clamped generative hyphae, and preference for gymnosperms (Núñez and Ryvarden 1997). In the ITS phylogenetic tree, *A. tenuissimus* formed a clade with *Xylobolus frustulatus* (Fig. 1). Except for producing amyloid basidiospores, they are distinctly different in basidiocarp morphology and substrate preferences.

Aleurodiscus tropicus clustered with *A. cerussatus* with high BT and BPPs values in the ITS phylogenetic tree (Fig. 1). Morphologically, they have smooth basidiospores of similar size, gloeocystidia, and numerous acanthophyses. *Aleurodiscus cerussatus*, however, has clamped generative hyphae, basidia without lateral protuberances, and grows on woody gymnosperms and angiosperms in north temperate regions

(Núñez and Ryvarden 1997). In addition to *A. tropicus*, *A. andinus* Núñez & Ryvarden and *A. thoenii* (Boidin, Lanq. & Gilles) Núñez & Ryvarden, also occur on bamboo and possess smooth basidiospores. *Aleurodiscus andinus* differs from *A. tropicus* in lacking acanthophyses, having basidia that lack acanthophysoid appendages, developing slightly larger basidiospores (12–14 × 7–8 μm), and with a South American distribution, whereas *Aleurodiscus thoenii* has clamped generative hyphae and also lack acanthophysoid appendages on basidia (Núñez and Ryvarden 1997).

Species of *Aleurodiscus* s.l. occur on a variety of substrates, such as ferns, grass, gymnosperms and angiosperms (Núñez and Ryvarden 1997). Although most species of *Aleurodiscus* s.l. are saprobes, some occur on bark of living trees and have a strong host preference. For example, *A. ljubarskii* Parmasto and *A. oakesii* (Berk. & M.A. Curtis) Pat. occur primarily on *Quercus*, whereas *A. tsugae* Yasuda occurs on *Pinus*.

There are fewer than 100 corticioid fungal species recorded worldwide on just a few species of woody bamboos. The six species of *Aleurodiscus* s.l. on bamboo represent about 7% of all the known species of *Aleurodiscus* s.l. We anticipate that additional species of *Aleurodiscus* s.l. and other corticioid wood-decay fungi on bamboo will be discovered as more studies in tropical areas are conducted.

Key to species of *Aleurodiscus* s.l. on bamboo worldwide

1. Basidiospores smooth..... 2
1. Basidiospores ornamented..... 4
2. Generative hyphae with clamp connections..... *A. thoenii*
2. Generative hyphae with simple septa..... 3
3. Acanthophyses absent; basidia smooth; distributed in South America..... *A. andinus*
3. Acanthophyses present; basidia with an acanthophysoid appendage; distributed in southern tropical Asia..... *A. tropicus*
4. Basidiospores subglobose to globose..... *A. bambusicola*
4. Basidiospores ellipsoid..... 5
5. Generative hyphae with clamp connections, acanthophyses present..... *A. phragmitis*
5. Generative hyphae with simple septa, acanthophyses absent..... *A. tenuissimus*

Disclosure

We declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of P.R. China.

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