



## *Lentithecium cangshanense* sp. nov. (Lentitheciaceae) from freshwater habitats in Yunnan Province, China

HONG-YAN SU<sup>1,2</sup>, ZONG-LONG LUO<sup>2,3</sup>, XIAO-YING LIU<sup>2,4</sup>, XI-JUN SU<sup>2</sup>, DIAN-MING HU<sup>5</sup>, DE-QUN ZHOU<sup>1\*</sup>, ALI H. BAHKALI<sup>6</sup> & KEVIN D. HYDE<sup>3,6</sup>

<sup>1</sup>Faculty of Environmental Sciences & Engineering, Kunming University of Science & Technology, Kunming 650500, Yunnan, China.

<sup>2</sup>College of Agriculture & Biological Sciences, Dali University, Dali 671003, Yunnan, China.

<sup>3</sup>Centre of Excellence in Fungal Research, and School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand.

<sup>4</sup>College of Basic Medicine, Dali University, Dali 671000, Yunnan, China.

<sup>5</sup>College of Bioscience & Engineering, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, China.

<sup>6</sup>Department of Botany & Microbiology, King Saud University, Riyadh, Saudi Arabia

\*Corresponding author: De-qun Zhou, email address: 1549284671@qq.com.

### Abstract

*Lentithecium cangshanense* sp. nov. (Lentitheciaceae, Dothideomycetes), was found on submerged decaying wood in a freshwater stream in Yunnan Province, China. The species is characterized by its black, semi-immersed to superficial, globose ascomata, cylindrical or obclavate, short pedicellate, bitunicate asci and bi-seriate, fusiform, 1-septate, yellowish to brown ascospores. Phylogenetic analyses of combined LSU, SSU and RPB2 sequence data show that *L. cangshanense* belongs in the family Lentitheciaceae, order Pleosporales and is a distinct species in the genus. The new species is introduced with an illustrated account and compared with morphologically and phylogenetically similar species.

**Key words:** aquatic fungi, Lentitheciaceae, phylogeny, taxonomy

### Introduction

Freshwater lignicolous fungi, are mostly ascomycetes, and have been collected and described from wood submerged in streams (Wong *et al.* 1998, Cai *et al.* 2003a, b, Jones & Choeyklin 2008, Hyde *et al.* 1998a, 1999, 2015) and lakes (Hyde *et al.* 1998b, Luo *et al.* 2004), and in surveys in Australia, China, Malaysia, Philippines and Thailand (Nawawi 1985, Kuthubutheen 1987, Hyde 1995, Sivichai *et al.* 2000, Cai *et al.* 2003, Jones *et al.* 2007, Kurniawati *et al.* 2010, Luo *et al.* 2015, 2016). The results of some of these studies have been summarized by Hyde *et al.* (2016). Two hundred and fifty-six freshwater ascomycetes have been reported from China including 57 Dothideomycetes with many species are from Yunnan Province (Hu *et al.* 2013). Although there have been several investigations on freshwater fungi in Yunnan Province (Cai *et al.* 2002, Jeewon *et al.* 2003, Luo *et al.* 2004, Shen & Ye 2006, Hu *et al.* 2013), studies in new streams generally reveal novel taxa (Liu *et al.* 2015, Su *et al.* 2015).

Zhang *et al.* (2012) introduced the family Lentitheciaceae to accommodate massarina-like species in the suborder Massarineae (Zhang *et al.* 2009, 2012, Hyde *et al.* 2013). Presently, there are eight genera included in Lentitheciaceae *viz.* *Darksidea*, *Katumotoa*, *Keissleriella*, *Lentithecium*, *Murilentithecium*, *Poaceascoma*, *Setoseptoria* and *Tingoldiogo*, with *Lentithecium fluviatile* (Aptroot & Van Ryck.) K.D. Hyde *et al.* being the type (Hyde *et al.* 2013, Phookamsak *et al.* 2015, Knapp *et al.* 2015). Members of Lentitheciaceae were reported as being saprobes on herbaceous and woody plants and produce coelomycetous asexual morphs.

*Lentithecium* was introduced by Zhang *et al.* (2009) to accommodate the species which share similar morphological characters in having immersed to erumpent lenticular ascomata, clavate asci, anastomosing cellular pseudoparaphyses and hyaline 1-septate ascospores. There are seven *Lentithecium* names in Index Fungorum (2016).

In this paper, we introduce a new ascomycetes from Cangshan Mountain, western of Yunnan Province, with an illustrated account and its novelty is supported by morphological and molecular data.

## Materials and methods

### *Isolation and morphology*

Submerged decaying wood samples were collected from a stream in Cangshan Mountain, Yunnan Province, China in July 2013 and returned to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. The samples were processed and examined following the methods described by Taylor & Hyde (2003). Morphological observations were made using a Motic SMZ 168 Series stereomicroscope and photographed using an OLYMPUS BX51 microscope-camera system. The fungal structures were measured using Image-Pro-Express software.

Single spore isolations were made to obtain pure cultures as described in Chomnunti *et al.* (2014). Herbarium specimens are deposited in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. The pure cultures are deposited in Kunming Institute of Botany Culture Collection (KUMCC) and Dali University Culture Collection (DLUCC). Facesoffungi numbers were obtained as in Jayasiri *et al.* (2015).

### *DNA extraction, PCR amplification and sequencing*

Total genomic DNA was extracted from fresh fungal mycelium grown on PDA at 25 °C. The EZ gene™ Fungal gDNA Kit (GD2416) was used to extract DNA according to the manufacturer's instructions. The gene regions of the large subunit of the nuclear ribosomal LSU, SSU and protein coding RPB2 were amplified using the primer pairs LROR/LR7 (Vigalys & Hester 1990), NS1/NS4 (White *et al.* 1990) and fRPB2-5F/fRPB2-7cR (Liu *et al.* 1999) respectively. Polymerase chain reaction (PCR) was carried out using the following protocol: The final volume of the PCR reaction was 25 µL and contained 12.5 µL of 2×Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µL Taq DNA Polymerase, 500 µM dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl pH 8.3, 100mM KCl, 3 mM MgCl<sub>2</sub>, stabilizer and enhancer), 1 µL of each primer (10 µM), 1 µL genomic DNA extract and 9.5 µL deionised water. The PCR thermal cycle program for LSU and SSU amplifications were followed as: initially 95 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 40 seconds, elongation at 72 °C for 90 seconds, and final extension at 72 °C for 10 mins. The PCR thermal cycle program for the partial RNA polymerase second largest subunit (RPB2) was followed as initially 95 °C for 5 mins, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 2 mins, elongation at 72 °C for 90 seconds, and final extension at 72 °C for 10 mins. PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). The PCR products were observed on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were conducted at Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, P.R. China).

### *Phylogenetic analysis*

Sequences generated from this study were analyzed with reference sequences from GenBank and those derived from Knapp *et al.* (2015), Phookamsak *et al.* (2015), and Luo *et al.* (2016). The consensus sequences were initially aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) (Katoh & Standley 2013) and further improved using Bioedit v.5.0.6 (Hall 2001) and ClustalX v. 1.83 (Thompson *et al.* 1997) to allow maximum alignment and maximum sequence similarity.

A maximum likelihood analysis was performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMA substitution model with a final ln value of -20356.150570.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

Bayesian analyses were performed by using PAUP v.4.0b10 (Swofford 2002) and MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4 (Liu *et al.* 2012). Six simultaneous Markov Chains were run for 1 million generations and trees were sampled every 100<sup>th</sup> generations (Resulting 10000 total trees) (Cai *et al.* 2006). The first 2000 trees representing the

burn-in phase of the analyses were discarded and the remaining 8000 (post burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.* 2006, Liu *et al.* 2012 ).

Trees were viewed in Treeview (Page 1996). Sequences derived in this study are deposited in GenBank (Table 1).

**TABLE 1.** Isolates used in this study and their GenBank accession numbers, newly generated sequences are indicated in red.

Taxon	Culture/voucher	GenBank Accession Number		
		LSU	SSU	<i>RPB2</i>
<i>Bambusicola bambusae</i>	MFLUCC 11-0614	JX442035	JX442039	KP761718
<i>Bambusicola irregulisporea</i>	MFLUCC 11-0437	JX442036	JX442040	KP761719
<i>Bambusicola massarinia</i>	MFLUCC 11-0389	JX442037	JX442041	KP761716
<i>Bambusicola splendida</i>	MFLUCC 11-0439	JX442038	JX442042	KP761717
<i>Corynespora cassiicola</i>	CBS 100822	GU301808	GU296144	GU371742
<i>Corynespora smithii</i>	CABI 5649b	GU323201	–	GU371783
<i>Darksidea zeta</i>	CBS 135640	KP184013	KP184071	–
<i>Darksidea alpha</i>	CBS 135650	KP184019	KP184049	–
<i>Darksidea beta</i>	CBS 135637	KP184023	KP184074	–
<i>Darksidea epsilon</i>	CBS 135658	KP184029	KP184070	–
<i>Deniquelata barringtoniae</i>	MFLUCC 11-0422	JX254655	JX254656	–
<i>Falciformispora lignatilis</i>	BCC 21117	GU371826	GU371834	–
<i>Falciformispora lignatilis</i>	BCC 21118	GU371827	GU371835	–
<i>Helicascus nypae</i>	BCC 36752	GU479789	GU479755	GU479827
<i>Kalmusia brevispora</i>	KT 1466	AB524600	AB524459	AB539099
<i>Kalmusia brevispora</i>	KT 2313	AB524601	AB524460	AB539100
<i>Karstenula rhodostoma</i>	CBS 690.94	GU301821	GU296154	GU371788
<i>Katumotoa bambusicola</i>	MAFF 239641	AB524595	AB524454	AB539095
<i>Keissleriella cladophila</i>	CBS 104.55	GU301822	GU296155	–
<i>Keissleriella dactylis</i>	MFUCC 13-0751	KP197668	KP197666	–
<i>Keissleriella poagena</i>	CBS 136767	KJ869170	–	–
<i>Keissleriella trichophoricola</i>	CBS 136770	KJ869171	–	–
<i>Lentithecium aquaticum</i>	CBS 123099	GU301823	GU296156	–
<i>Lentithecium arundinaceum</i>	CBS 619.86	GU301824	GU296157	–
<i>Lentithecium cangshanense</i>	DLUCC 0143	KU991149	KU991150	KU991151
<i>Lentithecium fluviatile</i>	CBS 122367	GU301825	GU296158	–
<i>Massarina cisti</i>	CBS 266.62	FJ795447	FJ795490	FJ795464
<i>Massarina eburnea</i>	CBS 473.64	GU301840	GU296170	GU371732
<i>Melanomma pulvis-pyrius</i>	CBS 124080	GU456323	GU456302	GU456350
<i>Montagnula opulenta</i>	CBS 168.34	DQ678086	AF164370	DQ677984
<i>Morosphaeria ramunculicola</i>	JK 5304B	GU479794	GU479760	GU479831
<i>Murilentithecium clematidis</i>	MFLUCC 14-0562	KM408760	KM408761	KM454447
<i>Murilentithecium clematidis</i>	MFLUCC 14-0561	KM408758	KM408760	KM454446
<i>Neottiosporina paspali</i>	CBS 331.37	EU754172	EU754073	GU371779
<i>Ophiosphaerella sasicola</i>	MAFF 239644	AB524599	AB524458	–
<i>Palmiascoma gregariascomum</i>	MFLUCC 11-0175	KP744495	KP753958	–

... Continued on next page

TABLE 1. (Continued)

Taxon	Culture/voucher	GenBank Accession Number		
		LSU	SSU	RPB2
<i>Poaceascoma helicoides</i>	MFLUCC 11-0136	KP998462	KP998463	KP998460
<i>Poaceascoma aquaticum</i>	MFLUCC 14-0048	KT324690	KT324691	KT373846
<i>Setoseptoria phragmitis</i>	CBS 114802	KF251752	–	–
<i>Stagonospora macropycnidia</i>	CBS 114202	GU301873	GU296198	–
<i>Stagonospora paludosa</i>	CBS 135088	KF251760	–	KF252262
<i>Tingoldiagio graminicola</i>	JCM 16485	AB521743	AB521726	–
<i>Trematosphaeria pertusa</i>	CBS 122368	FJ201990	FJ201991	FJ795476
<i>Trematosphaeria pertusa</i>	CBS 122371	FJ201992	FJ201993	GU371801

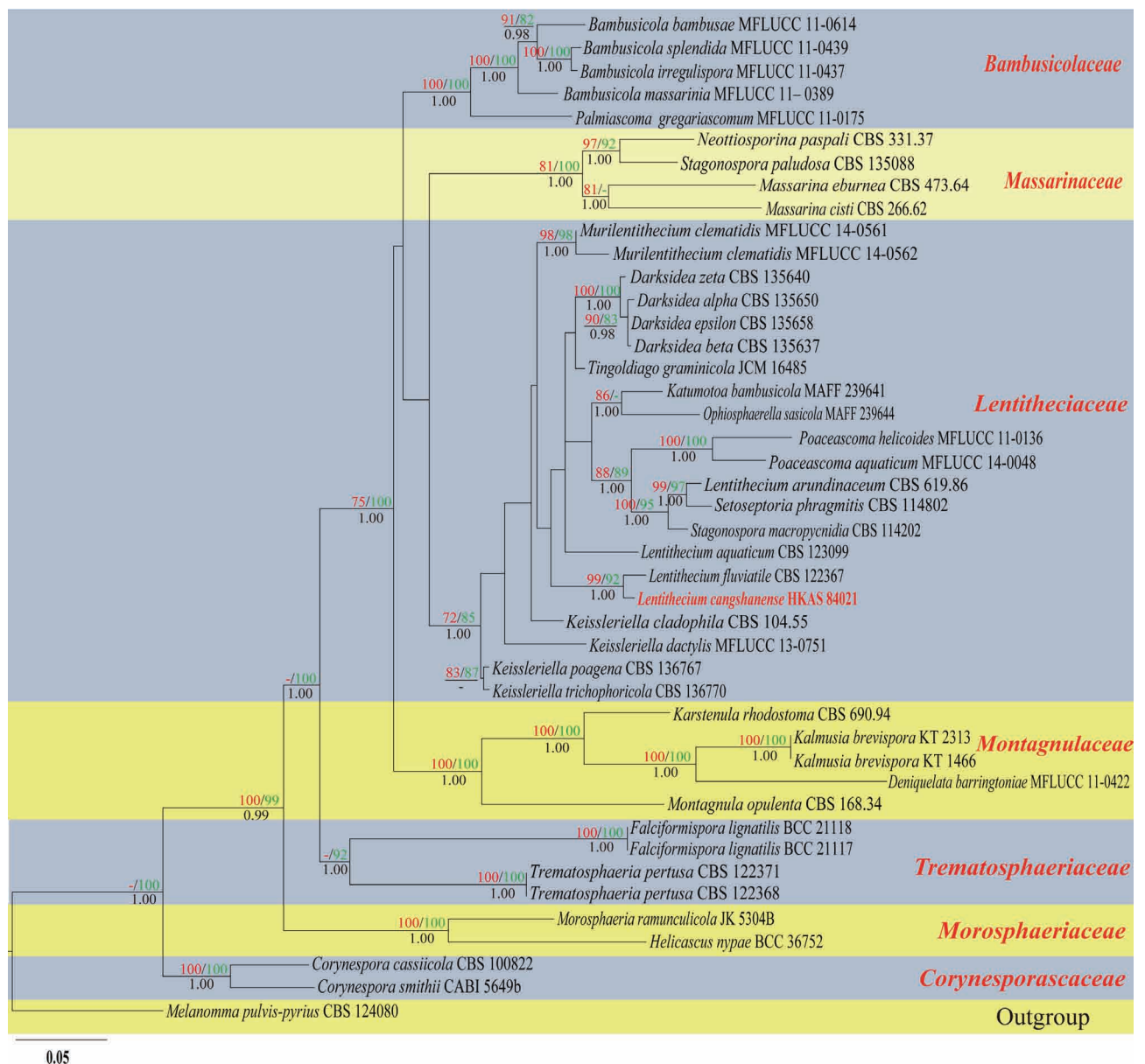


FIGURE 1. Phylogram generated from maximum likelihood analysis (RAxML) based on combined LSU, SSU and RPB2 sequenced data of the families in the suborder Massarinae. Bootstrap support values for maximum likelihood (ML, red) and maximum parsimony (MP, green) equal to or greater than 70% are given above the nodes. The values of the Bayesian posterior probabilities from MCMC analyses (BYPP, black) equal or higher than 95% are given below the nodes. The tree is rooted to *Melanomma pulvis-pyrius* (CBS 124080). Newly generated sequences are indicated in red.

## Results

### Phylogeny

Analysis of combined LSU, SSU and RPB2 sequence data were used to determine the taxonomic placement of our strain. The dataset comprised 44 taxa included all of genera of Lentitheciaceae, with *Melanomma pulvis-pyrius* (Pers.) Fuckel (CBS 124080) as the outgroup taxon. The phylogenetic trees generated by Maximum likelihood (ML), Maximum-parsimony (MP) and Bayesian analyses of combined LSU, SSU, RPB2 sequence data shown our strain to cluster in the family Lentitheciaceae (FIG. 1). The best scoring RAxML tree was selected to represent the relationships among the taxa and is shown in FIG. 1. The phylogenetic trees generated by Maximum likelihood (ML), Maximum-parsimony (MP) and Bayesian analyses of combined LSU, SSU, RPB2 gene regions showed that our strains clustered with *Lentithecium fluviatile* in the genus *Lentithecium* (FIG. 1).

### Taxonomy

*Lentithecium cangshanense* Z.L. Luo, X.J. Su & K.D. Hyde, *sp. nov.* **FIGURE 2.**

Index Fungorum: IF 552050; Facesoffungi number: FoF 02079.

Etymology: With reference to the collecting location, Cangshan Mountain.

Holotype: HKAS 84021.



**FIGURE 2.** *Lentithecium cangshanense* (HKAS 84021, holotype). **a, b.** Ascomata on submerged bamboo. **c.** Section through ascoma. **d.** Pseudoparaphyses. **e.** Section of peridium. **f–h.** Asci. **i–n.** Ascospores. **o.** Germinating ascospore. Scale bars: **c** = 150  $\mu$ m, **e** = 100  $\mu$ m, **f–h** = 25  $\mu$ m, **o** = 20  $\mu$ m, **d, i–n** = 10  $\mu$ m.

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph:** *Ascomata* 210–310  $\mu$ m high, 220–320  $\mu$ m in diam, scattered, black, immersed to partially erumpent, globose to subglobose, glabrous, with papilla visible as raised, dark spots on host surface, ostiole central. *Peridium* 20–29  $\mu$ m wide, of equal thickness, composed of several layers of

pseudoparenchymatous cells, arranged in a *textura angularis*, pigmented at the outer part. *Hamathecium* comprising numerous, 2.5–3.5 µm wide, septate pseudoparaphyses, embedded in a mucilaginous matrix, rarely anastomosing between and above the asci. *Asci* 65–78 × 11–13 µm ( $\bar{x}$  = 71 × 12 µm, n = 20), cylindric-clavate, pedicellate, apically rounded, with an ocular chamber. *Ascospores* 16.5–17.5 × 6–7 µm ( $\bar{x}$  = 17 × 6.5 µm, n = 20), bi-seriate, hyaline to subhyaline when young and pale brown to brown when mature, broadly fusiform, inequilateral in side view, 1-septate, slightly constricted at the septum, upper cell wider, smooth-walled, with four refractive globules. *Asexual morph*: Undetermined.

**Material examined:**—CHINA, Yunnan Province (N 25°50'52.39", E 100°05'59.87"), saprobic on decaying wood submerged in a stream, July 2013, Z.L. Luo, S-143 (HKAS 84021, **holotype**), ex-type culture, DLUCC 0143 = KUMCC; *ibid.* (DLU14–143, **isotype**).

Notes: *Lentithecium cangshanense* is typical of *Lentithecium* being characterized by scattered, semi-immersed to superficial, ascomata, septate pseudoparaphyses, bitunicate, pedicellate asci, bi-seriate, pale brown to brown ascospores which are slightly constricted at the septum. *Lentithecium cangshanense* resembles *L. aquaticum* in having scattered ascomata, a peridium of *textura prismatica* and broadly fusiform, ascospores which are in equilateral in side view, and have four refractive globules and slightly constricted at the septum. However, *L. cangshanense* differs from *L. aquaticum* in having a thicker, pale black to hyaline peridium, narrower and shorter asci ( $\bar{x}$  = 71.5 × 12 µm, versus 175 × 21 µm) and smaller ascospores ( $\bar{x}$  = 17 × 6.5 µm, versus 28 × 10.5 µm). In addition, the molecular analysis showed that this new fungus is clusters with *L. fluviatile* with strong support (FIG. 1). However, they have different morphological characters, *L. cangshanense* differs from *L. fluviatile* in having pale brown to brown, smaller ascospores (16.5–17.5 × 6–7 µm, versus 24–31 × 7–10 µm).

## Discussion

Zhang *et al.* (2012) introduced the family Lentitheciaceae in the order Pleosporales, typified by *Lentithecium*, with *L. fluviatile* as the type species. Species of the Lentitheciaceae occur on herbaceous plants such as *Phragmites* (*Lentithecium fluviatile*, *L. arundinaceum* (Sowerby) K.D. Hyde *et al.*, *Tingoldiogo graminicola* K. Hirayama & Kaz. Tanaka) and on submerged wood (*Lentithecium aquaticum* Ying Zhang *et al.*) in freshwater environments (Zhang *et al.* 2012). Phookamsak *et al.* (2015) introduced a new genus *Poaceascoma* to accommodate the ophiophora-like taxa in Lentitheciaceae and mentioned that the scolecospore taxa were polyphyletic in the class Dothideomycetes. Several clades in Lentitheciaceae are not well-resolved, future studies in this family is likely to collect and provide data for this undersampled group.

## Acknowledgements

We would like to thank the National Natural Science Foundation of China (Project ID: 31460015) and Middle-younger academic leaders of candidates projects in Yunnan Province (2012HB-042) for financial and laboratory support. Z.L. Luo thanks Shaun Pennycook (Landcare Research, New Zealand) for advising on the taxon name, K.D. Hyde is visiting Professor of King Saud University.

## References

- Ariyawansa, H.A., Hyde, K.D., Jayasiri, S.C., Buyck, B., Chethana, K.W.T., Dai, D.Q., Dai, Y.C., Daranagama, D.A., Jayawardena, R.S., Lücking, R., Ghobad-Nejhad, M., Niskanen, T., Thambugala, K.M., Voigt, K., Zhao, R.L., Li, G.J., Doilom, M., Boonmee, S., Yang, Z.L., Cai, Q., Cui, Y.Y., Bahkali, A.H., Chen, J., Cui, B.K., Chen, J.J., Dayarathne, M.C., Dissanayake, A.J., Ekanayaka, A.H., Hashimoto, A., Hongsanan, S., Jones, E.B.G., Larsson, E., Li, W.J., Li, Q.R., Liu, J.K., Luo, Z.L., Maharachchikumbura, S.S.N., Mapook, A., McKenzie, E.H.C., Norphanphoun, C., Konta, S., Pang, K.L., Perera, R.H., Phookamsak, R., Phukhamsakda, C., Pinruan, U., Randrianjohany, E., Singtripop, C., Tanaka, K., Tian, C.M., Tibpromma, S., Abdel-Wahab, M.A., Wanasinghe, D.N., Wijayawardene, N.N., Zhang, J.F., Zhang, H., Abdel-Aziz, F.A., Wedin, M., Westberg, M., Ammirati, J.F., Bulgakov, T.S., Lima, D.X., Callaghan, T.M., Callac, P., Chang, C.H., Coca, L.F., Dal-Forno, M., Dollhofer, V., Fliegerová, K., Greiner, K., Griffith, G.W.,

- Ho, H.M., Hofstetter, V., Jeewon, R., Kang, J.C., Wen, T.C., Kirk, P.M., Kytövuori, I., Lawrey, J.D., Xing, J., Li, H., Liu, Z.Y., Liu, X.Z., Liimatainen, K., Lumbsch, H.T., Matsumura, M., Moncada, B., Nuankaew, S., Parnmen, S., Santiago, A.L.C.M.A., Sommai, S., Song, Y., Souza, C.A.F., Souza-Motta, C.M., Su, H.Y., Suetrong, S., Wang, Y., Wei, S.F., Yuan, H.S., Zhou, L.W., Réblová, M., Fournier, J., Camporesi, E., Luangsa-ard, J.J., Tسانathai, K., Khonsanit, A., Thanakitpipattana, D., Somrithipol, S., Diederich, P., Millanes, A.M., Common, R.S., Stadler, M., Yan, J.Y., Li, X.H., Lee, H.W., Nguyen, T.T.T., Lee, H.B., Battistin, E., Marsico, O., Vizzini, A., Vila, J., Ercole, E., Eberhardt, U., Simonini, G., Wen, H.A. & Chen, X.H. (2015b). Fungal Diversity Notes 111–252 - Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 75 (1): 27–274.  
<http://dx.doi.org/10.1007/s13225-015-0346-5>
- Cai, L., Tsui, C., Zhang, K.Q. & Hyde, K.D. (2002) Aquatic fungi from Lake Fuxian, Yunnan, China. *Fungal Diversity* 9: 57–70.
- Cai, L., Zhang, K.Q., McKenzie, E.H.C. & Hyde, K.D. (2003) Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. *Fungal Diversity* 13: 1–12.
- Cai, L., Jeewon, R. & Hyde, K.D. (2006) Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* 110: 137–150.  
<http://dx.doi.org/10.1016/j.mycres.2005.09.014>
- Chomnunti, P., Hongsanan, S., Aguirre-Hudson, B., Tian, Q., Peršoh, D., Dhimi, M.K., Alias, A.S., Xu, J.C., Liu, X.Z., Stadler, M. & Hyde, K.D. (2014) The sooty moulds. *Fungal Diversity* 66 (1): 1–36.  
<http://dx.doi.org/10.1007/s13225-014-0278-5>
- Hall, T. (2001) BioEdit version 5.0. 6. North Carolina State University, Department of Microbiology, Raleigh, North Carolina 192.
- Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182.  
<http://dx.doi.org/10.1093/sysbio/42.2.182>
- Hu, D.M., Liu, F. & Cai, L. (2013) Biodiversity of aquatic fungi in China. *Mycology* 4 (3): 125–168.  
<http://dx.doi.org/10.1080/21501203.2013.835752>
- Hyde, K.D. (1995) Tropical Australia freshwater fungi. VII. New genera and species of ascomycetes. *Nova Hedwigia* 61: 119–140.
- Hyde, K.D., Goh, T.K. & Steinke, T.D. (1998a) Fungi on submerged wood in the Palmiet River, Durban, South Africa. *South African Journal of Botany* 64: 151–162.  
[http://dx.doi.org/10.1016/S0254-6299\(15\)30860-7](http://dx.doi.org/10.1016/S0254-6299(15)30860-7)
- Hyde, K.D. & Goh, T.K. (1998b) Fungi on submerged wood in Lake Barrine, north Queensland, Australia. *Mycological Research* 102: 739–749.  
<http://dx.doi.org/10.1017/S0953756297005868>
- Hyde, K.D. & Goh, T.K. (1999) Fungi on submerged wood from the River Coln, the Cotswolds, UK. *Mycological Research* 103: 1561–1574.  
<http://dx.doi.org/10.1017/S0953756299008989>
- Hyde, K.D., Jones, E.B.G., Liu, J.K., Ariyawansa, H., Boehm, E., Boonmee, S., Braun, U., Chomnunti, P., Crous, P.W., Dai, D.Q., Diederich, P., Dissanayake, A., Doilom, M., Doveri, F., Hongsanan, S., Jayawardena, R., Lawrey, J.D., Li, Y.M., Liu, Y.X., Lücking, R., Monkai, J., Muggia, L., Nelsen, M.P., Pang, K.L., Phookamsak, R., Senanayake, I., Shearer, C.A., Suetrong, S., Tanaka, K., Thambugala, K.M., Wijayawardene, N.N., Wikee, S., Wu, H.X., Zhang, Y., Aguirre-Hudson, B., Alias, S.A., Aptroot, A., Bahkali, A.H., Bezerra, J.L., Bhat, D.J., Camporesi, E., Chukeatirote, E., Gueidan, C., Hawksworth, D.L., Hirayama, K., Hoog, S.D., Kang, J.C., Knudsen, K., Li, W.J., Li, X.H., Liu, Z.Y., Mapook, A., McKenzie, E.H.C., Miller, A.N., Mortimer, P.E., Phillips, A.J.L., Raja, H.A., Scheuer, C., Schumm, F., Taylor, J.E., Tian, Q., Tibpromma, S., Wanasinghe, D.N., Wang, Y., Xu, J.C., Yan, J.Y., Yacharoen, S. & Zhang, M. (2013) Families of Dothideomycetes. *Fungal Diversity* 63: 1–313.  
<http://dx.doi.org/10.1007/s13225-013-0263-4>
- Hyde, K.D., Fryar, S., Tian, Q., Bahkali, A.H. & Xu, J.C. (2016) Lignicolous freshwater fungi along a north-south latitudinal gradient in the Asian/Australian region; can we predict the affects of global warming on biodiversity and function? *Fungal Ecology* 19: 190–200.  
<http://dx.doi.org/10.1016/j.funeco.2015.07.002>
- Index Fungorum (2016) Index Fungorum. Available from: <http://www.indexfungorum.org/names/Names.asp> (accessed 1 June 2016)
- Jayasiri, S.C., Hyde, K.D., Ariyawansa, H.A., Bhat, J., Buyck, B., Cai, L., Dai Y.C., Abd-Elsalam, K.A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E.B.G., Bahkali, A.H., Karunarathna, S.C., Liu, J.K., Luangsa-ard, J.J., Lumbsch, H.T., Maharachchikumbura, S.S.N., McKenzie, E.H.C., Moncalvo, J.M., Ghobad-Nejhad, M., Nilsson, H., Pang, K.L., Pereira, O.L., Phillips, A.J.L., Raspé, O., Rollins, A.W., Romero, A.I., Etayo, J., Selçuk, F., Stephenson, S.L., Suetrong, S., Taylor, J.E., Tsui, C.K.M., Vizzini, A., Abdel-Wahab, M.A., Wen, T.C., Boonmee, S., Dai, D.Q., Daranagama, D.A., Dissanayake, A.J., Ekanayaka, A.H., Fryar, S.C., Hongsanan, S., Jayawardena, R.S., Li, W.J., Perera, R.H., Phookamsak, R., Silva, N.I., Thambugala, K.M., Tian, Q., Wijayawardene, N.N., Zhao, R.L., Zhao, Q., Kang, J.C. & Promputtha, I. (2015) The Faces of Fungi database: fungal names linked with morphology, phylogeny

- and human impacts. *Fungal diversity* 74 (1): 3–18.  
<http://dx.doi.org/10.1007/s13225-015-0351-8>
- Jeewon, R., Cai, L., Liew, E.C.Y., Zhang, K.Q. & Hyde, K.D. (2003) *Dyrithiopsis lakefuxianensis* gen. et sp. nov. from Fuxian Lake, Yunnan, China, and notes on the taxonomic confusion surrounding Dyrithium. *Mycologia* 95: 911–920.  
<http://dx.doi.org/10.2307/3762019>
- Jones, E.B.G., Alias, S.A. & Nawawi, A. (2007) New fungi described from Malaysia. In: Jones EBG, Hyde KD, Vikineswary S (eds) *Malaysian fungal diversity*: 377–419.
- Jones, E.B.G. & Choeyklin, R. (2008) Ecology of marine and freshwater basidiomycetes. In: Boddy L, Frankland JC, van West P (eds) *Ecology of saprotrophic basidiomycetes*. Academic Press: 301–324.  
[http://dx.doi.org/10.1016/S0275-0287\(08\)80018-5](http://dx.doi.org/10.1016/S0275-0287(08)80018-5)
- Katoh, K. & Standley, D.M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30: 772–780.  
<http://dx.doi.org/10.1093/molbev/mst010>
- Knapp, D.G., Kovács, G.M., Zajta, E., Groenewald, J.Z. & Crous, P.W. (2015) Dark septate endophytic pleosporalean genera from semiarid areas. *Persoonia* 35: 87–100.  
<http://dx.doi.org/10.3767/003158515X687669>
- Kurniawati, E., Zhang, H., Chukeatirote, E., Sulistyowati, L., Moslem, M.A. & Hyde, K.D. (2010) Diversity of freshwater ascomycetes in freshwater bodies at Amphoe Mae Chan, Chiang Rai. *Cryptogamie Mycologie* 31: 323–331.
- Kuthubutheen, A.J. (1987) A new species of *Phalangiospora* and further observations on *P. constricta* from Malaysia. *Transactions of the British Mycological Society* 89: 414–430.  
[http://dx.doi.org/10.1016/S0007-1536\(87\)80135-3](http://dx.doi.org/10.1016/S0007-1536(87)80135-3)
- Liu, J.K., Phookamsak, R., Doilom, M., Wikee, S., Li, Y.M., Ariyawansa, H., Boonmee, S., Chomnunti, P., Dai, D.Q., Bhat, J.D., Romero, A.I., Zhuang, W.Y., Monkai, J., Jones, E.B.G., Chukeatirote, E., KO-KO, W.T., Zhao, Y.C., Wang, Y. & Hyde, K.D. (2012) Towards a natural classification of Botryosphaeriales. *Fungal Diversity* 57: 149–210.  
<http://dx.doi.org/10.1007/s13225-012-0207-4>
- Liu, Y.J., Whelen, S. & Hall, B.D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.  
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026092>
- Liu, X.Y., Udayanga, D., Luo, Z.L., Chen, L.J., Zhou, D.Q., Su, H.Y. & Hyde, K.D. (2015) Backbone tree for Chaetothyriales with four new species of *Minimelanolocus* from aquatic habitats. *Fungal Biology* 119 (11): 1046–1062.  
<http://dx.doi.org/10.1016/j.funbio.2015.08.005>
- Luo, Z.L., Maharachchikumbura, S.S.N., Liu, X.Y., Li, S.H., Chen, L.J., Su, H.Y., Zhou, D.Q. & Hyde, K.D. (2015) *Annulatasascus saprophyticus* sp. nov. and *Pseudoannulatasascus* gen. nov. to accommodate *Annulatasascus biatriisporus* (Annulatascales, Sordariomycetes) from Thailand. *Phytotaxa* 239 (2): 174–182.  
<http://dx.doi.org/10.11646/phytotaxa.239.2.6>
- Luo, Z.L., Bahkali, A.H., Liu, X.Y., Phookamsak, R., Zhao, Y.C., Zhou, D.Q., Su, H.Y. & Hyde, K.D. (2016) *Poaceascoma aquaticum* sp. nov. (Lentitheciaceae), a new species from submerged bamboo in freshwater. *Phytotaxa* 253 (1): 71–80.  
<http://dx.doi.org/10.11646/phytotaxa.253.1.5>
- Nawawi, A. (1985) Basidiomycetes with branched water-borne conidia. *Botanical Journal of the Linnean Society* 91: 51–60.  
<http://dx.doi.org/10.1111/j.1095-8339.1985.tb01134.x>
- Nylander, J.A.A. (2004) MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Page, R.D.M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer applications in the biosciences* 12: 357–358.
- Phookamsak, R., Li, W.J., Dai, D.Q., Singtripop, C. & Hyde, K.D. (2015) *Poaceascoma* gen et sp. nov., a new genus with scolecospores in Lentitheciaceae. *Cryptogamie Mycologie* 36 (2): 225–236.  
<http://dx.doi.org/10.7872/crym/v36.iss2.2015.225>
- Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–331.  
<http://dx.doi.org/10.1007/BF02338839>
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572.  
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Shen, Y.H. & Ye, D.H. (2006) Laboulbeniales. *Flora Fungorum Sinicorum* 28: 1–294.
- Silvestro, D. & Michalak, I. (2011) RAXML-GUI: a graphical front-end for RAXML. *Organisms Diversity and Evolution* 12: 335–337.



<http://dx.doi.org/10.1007/s13127-011-0056-0>

- Sivichai, S., Jones, E.B.G. & Hywel-Jones, N.L. (2000) Fungal colonisation of wood in a freshwater stream at Khao Yai National Park, Thailand. *Fungal Diversity* 5: 71–88.
- Su, H.Y., Udayanga, D., Luo, Z.L., Manamgoda, D.S., Zhao, Y.C., Yang, J., Liu, X.Y., Mckenzie, E.H.C. & Hyde, K.D. (2015) Hyphomycetes from aquatic habitats in Southern China: Species of *Curvularia* (Pleosporaceae) and *Phragmocephala* (Melanommataceae). *Phytotaxa* 226 (3): 201–216.  
<http://dx.doi.org/10.11646/phytotaxa.226.3.1>
- Swofford, D.L. (2003) PAUP\*: phylogenetic analysis using parsimony, version 4.0 b10.
- Taylor, J.E. & Hyde, K.D. (2003) Microfungi of tropical and temperate palms. *Fungal Diversity Research Series* 12: 1–459.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.  
<http://dx.doi.org/10.1093/nar/25.24.4876>
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 (8): 4238–4246.
- White, T., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*: 315–322.
- Wong, S.W., Hyde, K.D. & Jones, E.B.G. (1998) *Halosarpheia heteroguttulata* sp.nov. from submerged wood in streams. *Canadian Journal of Botany* 76: 1857–1862.  
<http://dx.doi.org/10.1139/b98-163>
- Zhang, Y., Wang, H.K., Fournier, J., Crous, P.W., Jeewon, R., Pointing, S.B. & Hyde, K.D. (2009) Towards a phylogenetic clarification of *Lophiostoma*, *Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38: 225–251.