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ORIGINAL ARTICLE

Morphological and molecular taxonomy of *Jahnula dianchia* sp. nov. (Jahnulales) from submerged wood in Dianchi Lake, Yunnan China

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Abstract

In this study, we report the discovery of *Jahnula dianchia* sp. nov., a freshwater lignicolous fungus belonging to the order Jahnulales (Dothideomycetes, Ascomycota), from a submerged woody substrate from Dianchi Lake, Yunnan, China. Morphologically, this new taxon differs from other species of the genus in ascospore morphology; the ascospores of the new species are dark brown with mammiform apices. We also provide a phylogenetic tree showing the molecular relationships between the new taxon with the previously accepted *Jahnula* species. The analysis of combined ITS, LSU, and SSU sequence dataset places *J. dianchia* within the Jahnulales sister to *J. sangamonensis*. The phylogeny also shows the placement of both strains of *J. dianchia* in a well-supported independent sub-clade.

Keywords Aliquandostipitaceae · Dothideomycetes · Morphology · New species · Phylogeny

Introduction

Jahnulales (Dothideomycetes), an order of freshwater lignicolous ascomycetes, was established by Pang et al. (2002). This order is phylogenetically related to the order Dothideales, Patellariales, and Pleosporales (Campbell et al. 2007). Most species of the Jahnulales occur on rotting or soft submerged corticated or decorticated wood (Inderbitzin et al. 2001; Pang et al. 2002; Shenoy et al. 2006, 2010; Campbell et al. 2007; Suetrong et al. 2011; Tanaka et al. 2015). The order Jahnulales is characterized by ascomata with peridial walls multilayered, composed of large cells, stalked and (or) sessile bitunicate asci, and one-septate ascospores with gelatinous sheaths or appendages (Pang et al. 2002; Campbell et al. 2007; Suetrong et al. 2011; Jones et al. 2015). Most authors accept two families in this order, Aliquandostipitaceae and Manglicolaceae (Suetrong et al. 2011; Hyde et al. 2013; Jones et al. 2015). The family, *Aliquandostipitaceae* within this order was proposed based on the genus *Aliquandostipite*, which contains only tropical species and is

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characterized by broad septate hyphae, globose to subglobose ascomata, and one-septate ascospores (Inderbitzin et al. 2001). Six genera are accommodated in this family including *Aliquandostipite*, *Jahnula* and *Megalohypha* (Kirschstein 1936; Inderbitzin et al. 2001; Ferrer et al. 2007), known by its sexual morph, whereas *Brachiosphaera*, *Speiropsis* and *Xylomyces* are exclusively asexual morph (Tubaki 1958; Descals et al. 1976; Goos et al. 1977; Campbell et al. 2007; Suetrong et al. 2011). The other family of the order, *Manglicolaceae*, also present cylindrical asci and appendaged ascospores (Suetrong et al. 2011). The *Manglicolaceae*, represented by the marine fungus *Manglicola guatemalensis* (type) and the terrestrial fungus *M. samuelsii*, has been classified as an independent family based on LSU and SSU rRNA sequence analyses (Kohlmeyer and Kohlmeyer 1971; Huhndorf 1994; Jones et al. 2015). In addition, it differs from the members of the Aliquandostipitaceae by the production of obtusely clavate to fusiform, stipitate ascomata bearing a broad ostiole and unequally one-septate ascospores (Suetrong et al. 2011).

The largest genus within Jahnulales, *Jahnula*, was introduced by Kirschstein (1936) and is typified by *Jahnula aquatica*. All 16 species of *Jahnula* have been reported from wood or decorticated wood in fresh-water habitats (Hawksworth 1984; Hyde 1993; Hyde and Goh 1998; Hyde and Wong 1999; Ho et al. 2002; Pang et al. 2002; Pinruan et al. 2002; Raja and Shearer 2006; Campbell et al. 2007; Raja et al. 2008; Sivichai and Boonyuen 2010; Suetrong et al. 2011; Fournier et al. 2015). The genus is currently polyphyletic, and *Jahnula* sensu stricto accommodates *Jahnula aquatica*, *J. granulosa*, *J. potamophila*, and *J. rostrata* (Suetrong et al. 2011).

We are carrying out a survey of freshwater fungi along a north south latitudinal gradient in China and Thailand (Luo et al. 2017; Hyde et al. 2016a). Members of Jahnulales are major fungal species colonizing substrates in freshwater habitats, and recent taxonomic studies in freshwater habitats have revealed new asexual species (Luo et al. 2017). In this study, we report on two collections of *Jahnula* in China. The specimens were studied morphologically to enable identification, and regions of complete ITS and partial LSU and SSU rRNA gene were analyzed to determine their phylogenetic affinity to previously known *Jahnula* species.

Materials and methods

Sample collection, morphological studies, and isolation

Submerged dead wood pieces were collected from the Dianchi Lake, Yunnan Province in China in October 2016 and brought to the laboratory in zip lock plastic bags. Incubation of specimens was performed as outlined by Vrijmoed (2000). Fruiting bodies were found growing on decayed wood in a sterile plastic box after 2 weeks and were subsequently isolated based on the method of Chomnunti et al. (2014). Morphological characters were examined using an Olympus SZ61 stereoscope, and ascomata were sectioned by free-hand with a razor-blade. These sections were examined by a Nikon ECLIPSE Ni compound microscope, and images were taken with a Canon EOS 600D digital camera. Measurements were made with Tarosoft® Image Frame Work program v. 0.9.7. The specimens are deposited in the Kunming Institute of Botany, Academia Sinica and duplicated in Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. *Facesoffungi* (FoF) and Index Fungorum (IF) numbers were registered as explained in Jayasiri et al. (2015) and Index Fungorum (2017) (http://www.indexfungorum.org/names/names.asp). New species are established based on the recommendations outlined by Jeewon and Hyde (2016).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted directly from mycelium using a *Trelief*™ Plant Genomic DNA Kit following the instructions of the manufacturer. The genomic DNA was amplified by using polymerase chain reaction (PCR) in a 25 μL reaction mixture. Regions of the internal transcribed spacers (ITS1–5.8S-ITS2), partial large subunit rRNA (LSU), and partial small subunit rRNA (SSU) were amplified using primer pair ITS5 and ITS4 (White et al. 1990), LROR and LR5 (Vilgalys and Hester 1990) and NS1 and NS4 (White et al. 1990) respectively. Each PCR reactions contained 12.5 μL of 2 × Power Taq PCR MasterMix, 10.5 μL ddH_2O, and 0.5 μL of each primer (10 μM); and 1 μL genomic DNA extract and amplifications were carried out in an Applied Biosystems 2720 thermocycler (Foster City, CA, USA) with the following profile: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at 50 °C (SSU) or 53 °C (ITS and LSU) for 50 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 8 min. The PCR products were visualized by loading 5 μL on 1% agarose electrophoresis gels and bands viewed in Gel documentation system. The PCR products were sent to a commercial sequencing provider Tsingke Company, Beijing, P.R. China.

Phylogenetic analysis

The quality of our amplified nucleotide sequences were checked by Finch TV version 1.4.0 (www.geospiza.com/finchtv), and subjected to the BLAST search under the National Center for Biotechnology Information (NCBI). Then the closest matches for our strains were retrieved from the NCBI database (Pang et al. 2002; Campbell et al. 2007;

Prihatini et al. 2008; Suetrong et al. 2011; Phookamsak et al. 2014; Wanasinghe et al. 2014; Ariyawansa et al. 2015; Hyde et al. 2016b). Sequences were aligned using the default setting of MAFFT v. 7.310 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2016), and manually corrected using Bioedit 7.0.9.0 (Hall 1999).

The phylogenetic analyses of combined gene regions were performed using maximum-likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP) methods. The evolutionary model was obtained using MrModeltest v. 2.3 (Nylander et al. 2008) under the Akaike Information Criterion (AIC) performed in PAUP v. 4.0b10.

The maximum-likelihood analysis was run or performed using RAxML-HPC v.8 on XSEDE in CIPRES Science Gateway (Stamatakis 2014; Miller et al. 2010, 2015) with 1000 rapid bootstrap replicates using the General Time Reversible model incorporating invariant sites and a gamma distribution (GTR + I + G).

Bayesian inference was implemented by MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003) with the best-fit model (GTR + I + G for ITS and LSU; SYM + I + G for SSU) of sequence evolution estimated with MrModeltest 2.3 (Nylander et al. 2008). Posterior probabilities (PP) were estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001; Zhaxybayeva and Gogarten 2002; Rannala and Yang 2008). Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run from random trees for 100,000,000 generations and sampled every 1000 generations. The temperature value was lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01.

Phylogenetic analyses were also performed with maximum parsimony in PAUP v. 4.0b10 (Swofford 2002). Details are outlined as in Jeewon et al. (2002, 2003) and Cai et al. (2006). In brief, ambiguously aligned regions were excluded by hand and gaps were treated as missing data. Trees were inferred with the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited; branches of zero length were collapsed, and all parsimonious trees were saved. Tree length [TL], consistency index [CI], homoplasy index [HI], retention index [RI], and rescaled consistency index [RC] were calculated for the MP tree. Clade stability was assessed using a bootstrap (BT) analyses with 1000 replicates, each with ten replicates of random stepwise addition of taxa.

Phylogenetic trees were viewed with FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and processed by Adobe Illustrator CS5. Alignment and trees were deposited in TreeBASE (submission ID: 20896). The nucleotide sequence data of new taxa have been deposited in GenBank (Table 1).

Results

Phylogenetic analyses

The alignment comprised 2347 total characters including gaps. The tree was rooted with *Farlowiella carmichaeliana*. The Bayesian analysis resulted in a tree with the same topology and clades as the ML and MP trees. RAxML analysis yielded a best scoring tree with a final optimization likelihood value of − 16,010.965307 (Fig. 1). Parsimony analyses indicated that 1411 characters were constant; 264 variable characters were parsimony-uninformative, and 672 characters were parsimony-informative. The parsimony analysis of the data matrix resulted in a single most parsimonious tree (TL = 2691, CI = 0.544, RI = 0.751, RC = 0.409, HI = 0.456) is shown here. In the phylogenetic tree, the two isolates of *Jahnula dianchia* constitute a well-supported clade (100%, ML/1.0, PP/100%, MP) that is sister to *J. sangamonensis* (100%, ML/1.0, PP/100%, MP).

Taxonomy

Jahnula dianchia S.K. Huang & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF553200; *Facesoffungi number*: FoF 03149. Fig. 2

Etymology: The name *dianchia* refers to the geographic location were the specimen was collected.

Holotype: HKAS96327

Saprobic on dead wood. **Sexual morph**: *Ascomata* 307–418 × 248–374 μm ($\bar{x}$ = 380 × 325 μm, *n* = 5), perithecial, solitary, superficial to sub-immersed, unilocular, obpyriform to subglobose, dark brown to black, papillate, ostiolate. *Ostiole* central, short, lined with hyaline periphyses. *Peridium* 35–75 μm thick, membranous, composed of brown to hyaline cells of *textura angularis* (Fig. 2d). *Hamathecium* comprising 2–3 μm wide, septate, branched, filiform pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 198–238 × 16–20 μm ($\bar{x}$ = 217 × 19 μm, *n* = 20), 8-spored, bitunicate, fissitunicate, cylindrical, pedicellate, rounded at apex, with a distinct ocular chamber. *Ascospores* 24–32 × 10–21 μm ($\bar{x}$ = 27.5 × 13 μm, *n* = 50), uniseriate, initially hyaline, becoming dark brown at maturity, oval to broadly ellipsoid, 1-septate, with a mammiform apex, slightly curved, smooth to verruculose, multiguttulate, apiculate, rounded at lower end. **Asexual morph**: undetermined.

Culture characteristics: Ascospores germinating on potato dextrose agar (PDA) within 2 weeks at 23 °C, colony 1.5 cm diam., hyphae 3–10 μm thick, cream to faint yellow from above and reverse, with concentric zonation, with filamentous mycelium, filiform at margin, with rough surface and raised elevation.

Table 1 Strains and GenBank accession numbers of the isolates used in this study. Isolates from this study are in bold underline and the ex-type strains are in bold

Species	Voucher/Culture	GenBank accession number		
		ITS	LSU	SSU
Aliquandostipite crystallinus	**A514-1**	–	–	**EF175629**
A. crystallinus	F83-1	–	GU266239	GU266221
***A.** khaoyaiensis*	CBS 118232	AF201728	GU301796	AF201453
A. separans	CY2787	–	–	AF438179
A. siamensiae	SS81.02	–	EF175666	EF175645
A. sunyatsenii	**UBC-F13876**	**AF201727**	–	**AF201454**
Brachiosphaera tropicalis	E192-1	–	EF175653	GU266223
B. tropicalis	SS2523	FJ887923	JN819284	JN819287
Capnodium salicinum	CBS 131.34	AJ244240	EU019269	DQ677997
Cladosporium allicinum	AFTOL-ID 1591	–	DQ678074	DQ678022
Delphinella strobiligena	AFTOL-ID 1257	–	DQ470977	DQ471029
Dothidea sambuci	AFTOL-ID 274	DQ491505	AY544681	AY544722
Dothiora cannabinae	AFTOL-ID 1359	–	DQ470984	DQ479933
Elsinoe phaseoli	AFTOL-ID 1855	–	DQ678095	DQ678042
E. veneta	AFTOL-ID 1853	–	DQ767658	DQ767651
Farlowiella carmichaeliana	JX-43	KF836060	KF836062	KF836061
Jahnula aquatica	R68-1	JN942354	EF175655	EF175633
J. australiensis	SS0665	–	–	AF438182
J. bipileata	**F49-1**	**JN942353**	**EF175657**	**EF175635**
J. bipileata	AF220–1	JN942352	EF175656	EF175634
J. dianchia	**KUMCC 17-0034**	**KY928453**	**KY928454**	**KY928455**
J. dianchia	**KUMCC 17-0039**	**KY928456**	**KY928457**	**KY928458**
J. potamophila	F111-1	–	GU266242	GU266225
J. seychellensis	SS2113.2	–	EF175664	EF175643
J. seychellensis	A492	–	GU266243	EF175642
J. appendiculata	BCC11445	JN819279	FJ743445	FJ743439
J. appendiculata	BCC11400	JN819280	FJ743446	FJ743440
J. granulosa	SS1567	–	EF175659	EF175638
J. rostrata	F4-3	–	EF175660	GU266226
J. sangamonensis	F81-1	JN942351	EF175663	EF175641
J. sangamonensis	A482-1B	JN942350	EF175662	EF175640
Leptosphaeria biglobosa	UK28	DQ133893	–	–
L. maculans	UK7	DQ133891	–	–
Manglicola guatemalensis	BCC20157	JN819282	FJ743450	FJ743444
M. guatemalensis	BCC20156	JN819283	FJ743448	FJ743442
Megalohypha aqua-dulces	AF005-2a	–	EF175667	EF175646
M.aqua-dulces	AF005-2b	–	EF175668	–
Mycosphaerella punctiformis	AFTOL-ID 942	–	DQ470968	DQ471017
Myriangium duriaei	CBS 260.36	–	NG_027579	AF242266
M. hispanicum	CBS 247.33	–	GU301854	GU296180
Pseudoxylomyces elegans	SS1077	FJ887920	GU301796	AF201453
Scorias spongiosa	AFTOL-ID 1594	–	DQ678075	DQ678024
Setosphaeria rostrata	ATCC:32197	AF071342	–	U42487
Speiropsis pedatospora	SS2229	FJ887926	JN819285	JN819290
S. pedatospora	SS2236	FJ887927	–	–
Stylodothis puccinioides	CBS 193.58	–	NG_027594	–

Table 1 (continued)

Species	Voucher/Culture	GenBank accession number		
		ITS	LSU	SSU
Westerdykella cylindrica	ATCC 24077	AY943056	NG_027595	NG_016502
W. dispersa	CBS 71271	DQ468031	DQ468051	–
Xylomyces aquaticus	CBS636.91	FJ887921	–	–
X. chlamydosporus	SS0807	FJ887918	–	–
X. chlamydosporus	SS2917	FJ887919	–	JN819291

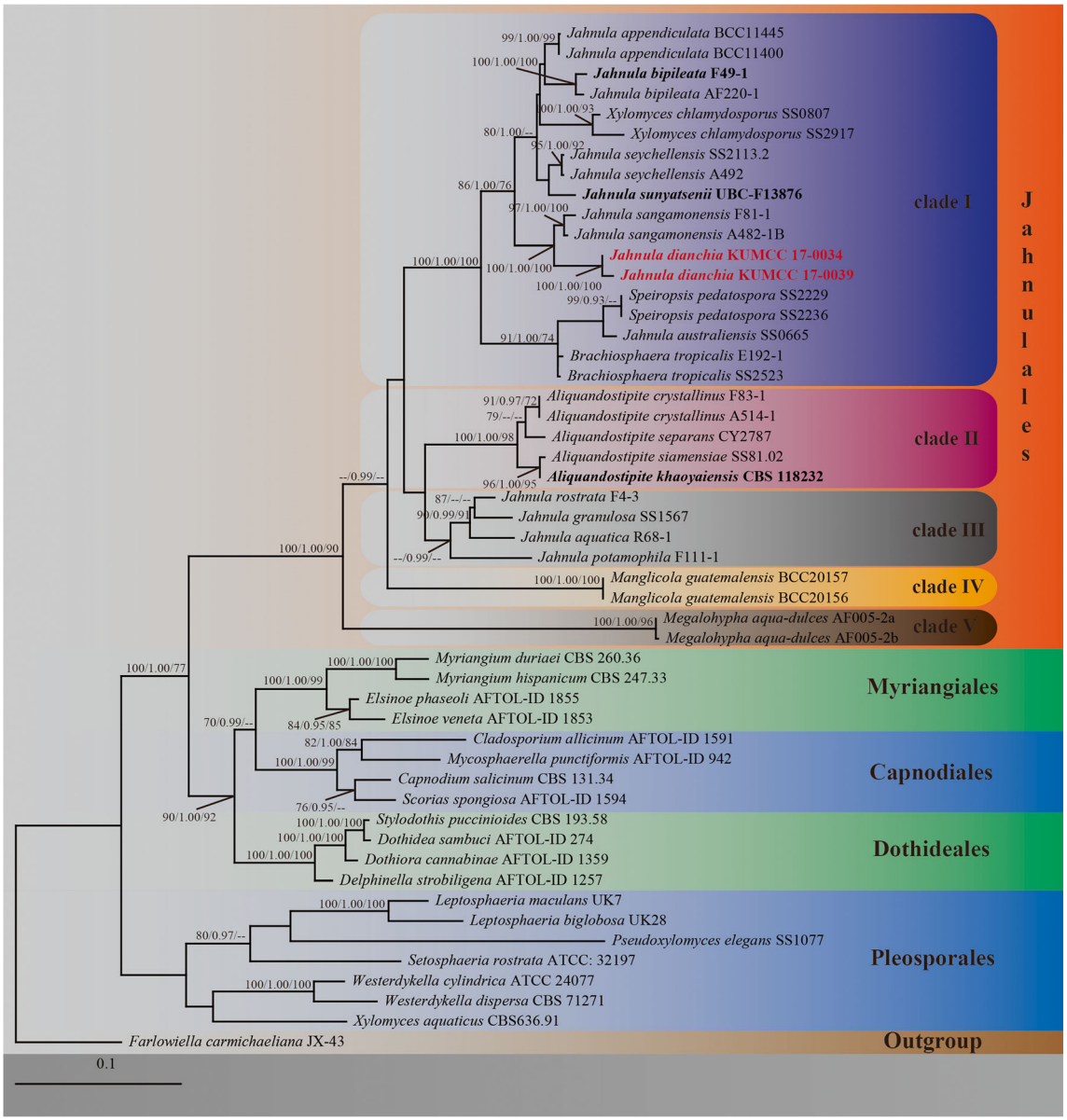

Fig. 1 Maximum likelihood phylogenetic tree generated from analysis of a combined ITS, LSU, and SSU sequences dataset for 51 taxa of Dothideomycetes and *Farlowiella carmichaeliana* as the outgroup taxon. ML support values greater than 70% (BSML, *left*), Bayesian posterior probabilities greater than 0.95 (BYPP, *middle*) and MP bootstrap value higher than 70% (BSMP, *right*) are indicated above the nodes. The strain numbers are noted after the species names. Ex-type strains are indicated in bold. Isolates from this study are indicated in red bold

Fig. 2 *Jahnula dianchia* (HKAS96327, holotype). **a** Material. **b** Ascoma on host. **c** Ascoma in vertical section. **d** Peridium. **e** Ostiole in vertical section with periphyses. **f** Asci with pseudoparaphyses. **g–i** Asci. **j–l** Ascospores. **m** Germinating ascospores. Note: **g–h** stained in Melzer's reagent, arrow showing mammiform ascospores apex. Scale bars c, d = 100 μm, e–i = 50 μm, j–m = 20 μm

Material examined: CHINA, Yunnan, Kunming, Dianchi Lake (24° 51′ 29.18″ N, 102° 39′ 58.19″ E); on dead wood, 1 October 2016; S.K. Huang (KUN HKAS 96327, **holotype**), ibid. (MFLU 17-0693, **isotype**); Ex-type KUMCC 17-0034, MFLUCC 17-0887. ibid. (KUN HKAS 97459, MFLU 17-0698; KUMCC 17-0039, MFLUCC 17-0891).

Key to species of *Jahnula* based on the sexual morph

1. Ascospores with appendages 2
1. Ascospores without appendages 10
2. Ascospores with long appendages arising from both poles 3
2. Ascospores surrounded by mucilaginous sheath 4
2. Ascospores with mucilaginous pads at both poles 7
3. Ascospores 47.5–55 × 23.5–26.5 μm[4] .....***Jahnula appendiculata***
3. Ascospores 17.5–20 × 5–6.5 μm[7] .....***J. morakotii***
4. Two types of ascospores morphologies[2] .....***J. systyla***
4. One type of ascospores morphology 5
5. Ascospores light brown[2] .....***J. potamophila***
5. Ascospores dark brown 6
6. Ascospores 26–37.5 × 15–18 μm[2] .....***J. granulosa***
6. Ascospores 32–45 × 12–15 μm[5] .....***J. rostrata***
7. There are two types of ascospores[2] .....***J. seychellensis***
7. There are only one type of ascospores 8
8. Ascospores dark brown[5] .....***J. bipileata***
8. Ascospores light brown 9

9. Asci cylindrical, ascospores uniseriate[2] .....***J. bipolaris***
9. Asci obclavate, ascospores biseriate[3, 8] ..***J. sunyatsenii***
10. Ascospores ellipsoid to fusiform.....11
10. Ascospores oval to oblong 14
11. Ascospores basal cell shorter than apical cell[6] .....***J. apiospora***
11. Ascospores symmetrical 12
12. Ascospores longer than 30 μm[5] .....***J. aquatica***
12. Ascospores shorter than 30 μm 13
13. Ascospores with tapering apices[2] .....***J. poonythii***
13. Ascospores with rounded apices[5] .....***J. sangamonensis***
14. Asci cylindrical 15
14. Asci obclavate[9] .....***J. purpurea***
15. Ascospores without mammiform apex, 19–30 × 6–8 μm[1] .....***J. australiensis***
15. Ascospores with mammiform apex, 24–32 × 10.5–21 μm .....***J. dianchia***

[1] Hyde (1993); [2] Hyde and Wong (1999); [3] Inderbitzin et al. (2001); [4] Pinruan et al. (2002); [5] Raja and Shearer (2006); [6] Raja et al. (2008); [7] Sivichai and Boonyuen (2010); [8] Suetrong et al. (2011); [9] Fournier et al. (2015)

Discussion

As part of our studies on freshwater fungi in the Yunnan Province of China, we collected a fungal species that is morphologically similar to the genus *Jahnula* or *Jahnula* spp. Species of *Jahnula* are predominantly collected or isolated from freshwater habitats (Hyde 1993; Hyde and Wong 1999; Tsui et al. 2000; Ho et al. 2001; Pang et al. 2002; Pinruan et al. 2002; Raja and Shearer 2006; Raja et al. 2008; Sivichai and Boonyuen 2010; Suetrong et al. 2011; Fournier et al. 2015). Further, the two specimens in this study fit well with the diagnosis of *Jahnula* as previously circumscribed, but are morphologically distinct from other *Jahnula* species such as to justify the description and naming of a new species. In addition, DNA sequence data support that the new taxon, *J. dianchia*, is phylogenetically distinct from other known species. Cai et al. (2002) reported *Jahnula poonythii* K.D. Hyde & S.W. Wong from Dianchi Lake, which may be an earlier misidentified collection of our new species. However, fusiform ascospores are seen in *J. poonythii* whereas they are broadly ellipsoidal to oval in *J. dianchia*.

The phylogeny (Fig. 1) reveals a close relationship of the newly collected fungus to *J. sangamonensis* which has been reported from decorticated submerged woody debris in the USA (Raja and Shearer 2006). *Jahnula dianchia* is similar to *J. sangamonensis* but differs in the following aspects: (i) most of the ascomata of *J. dianchia* are superficial and *J. sangamonensis* is sub-immersed in the wood, (ii) the peridium of *J. dianchia* is 35–75 μm wide with the inner wall layer of ostiole lined with hyaline periphyses while those of *J. sangamonensis* are 40–44 *μm* wide with the inner wall layer of ostiole lined with reddish brown periphyses, (iii) the asci of two taxa are different in that endoascus of *J. sangamonensis* extends up to 500 μm long in water, and (iv) the ascospores of *J. dianchia* are similar to those of *J. sangamonensis* except that these are with mammiform apex. Seventeen species are included in the genus *Jahnula* (Suetrong et al. 2010, 2011; Fournier et al. 2015). Ten of them are different from *J. dianchia* by having ascospores with a gelatinous sheath, pads, or appendages (viz. *Jahnula appendiculata*, *J. bipileata*, *J. bipolaris*, *J. granulosa*, *J. morakotii*, *J. potamophila*, *J. rostrata*, *J. seychellensis*, *J. sunyatsenii* and *J. systyla*) (Fournier et al. 2015; Hyde and Wong 1999; Pang et al. 2002; Pinruan et al. 2002; Sivichai and Boonyuen 2010). The shape of ascospores in *Jahnula apiospora*, *J. australiensis*, *J. poonythii*, and *J. purpurea* is fusiform or oblong while in our new taxon, it is oval to broadly ellipsoid (Fournier et al. 2015; Hyde and Wong 1999; Raja et al. 2008). *Jahnula dianchia* is also different from *J. aquatica*, the type species of *Jahnula*, collected from Germany and South Africa, in terms of ascospore length (> 30 μm) (Hyde and Wong 1999).

To further support our new taxon, we follow the recommendations by Jeewon and Hyde (2016). Comparison of the 463 base pairs (bp) across the nuclear ribosomal DNA ITS region, including the spacers ITS1 and ITS2 together with the 5.8S rRNA gene reveals that there are 20 bp (4.3%) differences when compared to *J. sangamonensis*. In the same way, comparison of the 862 bp of the 28S ribosomal RNA gene region reveals 11 bp (1.3%) difference compared to *J. sangamonensis* that justifies our new species.

Despite having only two strains herein, *J. dianchia* constitutes a strong monophyletic subclade with high support and therefore establishment of a new species is justified. The phylogeny generated herein (Fig. 1) agrees with other published studies in recovering *Jahnula* as a non-monophyletic group within the Jahnulales. Five clades are recognized: Clade I comprises species that belongs to *Jahnula* sensu *lato* with high statistical support (Campbell et al. 2007; Suetrong et al. 2011) together with those anamorphic genus *Speiropsis* (typified by *S. pedatospora*) and *Brachiosphaera* taxa (typified by *B. tropicalis*) (Suetrong et al. 2011). Clade II includes species of *Aliquandostipite*, while clade III comprises species of *Jahnula* sensu stricto such as *J. aquatica*, *J. granulosa*, *J. potamophila*, and *J. rostrata* (Campbell et al. 2007, Suetrong et al. 2011). Clade IV represents the genus *Manglicola* (Suetrong et al. 2010, 2011), whereas Clade V accommodates the genus *Megalohypha* (Raja et al. 2011).

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