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Morpho-molecular characterization of two novel amphisphaeriaceous species from Yunnan, China

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Abstract

Amphisphaeria yunnanensis sp. nov. and *Lepteutypa qujingensis sp. nov.* are introduced in this study from dead twigs collected from an evergreen broadleaf forest area in Yunnan Province, China. Both species have immersed, sub-globose ascomata and overlapping uniseriate asci and multi-guttulate, fusiform, brown ascospores. *Amphisphaeria yunnanensis* is distinguished among similar taxa in having long and narrow ostiole and comparatively small fusiform ascospores. *Lepteutypa qujingensis* is characterized by smaller ascomata and ascospores compare to other *Lepteutypa* species. Based on LSU-ITS phylogeny and macro-micro morphology, both species are placed in Amphisphaeriaceae. Morphological comparisons of the accepted species in *Amphisphaeria* and *Lepteutypa* are provided.

Keywords: 2 new species, Amphisphaeriaceae, phylogeny, taxonomy

Introduction

Amphisphaeriaceae G. Winter was introduced by Winter (1884–1886) with its type genus *Amphisphaeria* Ces. & De Not., and has been transferred between Amphisphaeriales D. Hawksw. & O.E. Erikss. and Xylariales Nannf. according to different author arguments (e.g. Senanayake *et al.* 2015, Jaklitsch *et al.* 2016). Kirk *et al.* (2008) accepted Amphisphaeriaceae under Xylariales with 32 genera and 499 species. Following consecutive morpho-molecular and evolutionary studies by Senanayake *et al.* (2015), Samarakoon *et al.* (2016) and Hongsanan *et al.* (2017) Amphisphaeriaceae was placed in Amphisphaeriales. The latest update of Sordariomycetes by Hyde *et al.* (2020) accepted Amphisphaeriaceae is in Amphisphaeriales (Xylariomycetidae O.E. Erikss. & Winka) including 17 families.

In a morpho-molecular study, Senanayake *et al.* (2015) accepted *Amphisphaeria* as the only genus in Amphisphaeriaceae while transferring other genera to different families in Xylariomycetidae, viz., Iodosphaeriaceae O. Hilber, Phlogicylindriaceae Senan. & K.D. Hyde and Sporocadaceae Corda (= Bartaliniaceae Wijayaw. *et al.*). Maharachchikumbura *et al.* (2016) and Jaklitsch *et al.* (2016) accepted only *Amphisphaeria* and *Lepteutypa* Petr. in Amphisphaeriaceae based on morpho-molecular studies. However, several outlines of fungi have been accepted three

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genera *Amphisphaeria*, *Griphosphaerioma* Höhn. and *Lepteutypa* in Amphisphaeriaceae (Wijayawardene *et al.* 2018, 2020, Hyde *et al.* 2020).

Amphisphaeria is typified by *A. umbrina* (Fr.) De Not. with coelomycetous asexual morph (Samuels *et al.* 1987, Barr 1990, Hyde *et al.* 1996, Kang *et al.* 1999a, b, 2002). *Amphisphaeria* species are characterized by unitunicate asci, J+ or J- subapical ring and two-celled, light brown to dark brown ascospores (Barr 1975, Wang *et al.* 2004). Based on morphological characterization, Wang *et al.* (2004) accepted 12 *Amphisphaeria* species from 170 type material examined, while other species were placed in other genera mostly in Dothideomycetes. In addition, several recent studies have also been focused on *Amphisphaeria* based only on morphology i.e. *A. doidgeae* Marinc. et al., and morpho-molecular studies i.e. *A. acericola* Senan. et al., *A. flava* Samarak. & K.D. Hyde, *A. mangrovei* Devadatha & V.V. Sarma, *A. sorbi* Senan. & K.D. Hyde and *A. thailandica* Samarak. & K.D. Hyde (Marincowitz *et al.* 2008, Liu *et al.* 2015, Phookamsak *et al.* 2019, Samarakoon *et al.* 2019, Senanayake *et al.* 2019). Eighteen *Amphisphaeria* species are accepted by Hyde *et al.* (2020) among >190 species epithets listed in Index Fungorum (http: www.indexfungorum.org).

As compared to *Amphisphaeria*, there is less attention on fresh collection and molecular data of *Griphosphaerioma* and *Lepteutypa*. Two *Griphosphaerioma* species as *G. kansensis* Ellis & Everh. and *G. zelkovicola* Yas. Ono & Tak. Kobay have been described so far with lack of molecular data (Ono & Kobayashi 2003).

Lepteutypa is typified by *L. fuckelii* G.H. Otth (Petrak *et al.* 1923). Jaklitsch *et al.* (2016) proposed a neotype for *L. fuckelii*, a new combination of *L. uniseptata* K.M. Tsui and a novel species, *L. sambuci* Jaklitsch & Voglmayr based on morpho-molecular study. Luo *et al.* (2019) introduced *L. aquatica* Z.L Luo *et al.* from freshwater habitat. Fifteen *Lepteutypa* species are listed in Index Fungorum **(**http: www.indexfungorum.org) and only five species (*L. aquatica, L. fuckeli, L. sambuci* and *L. uniseptata*) have sequence data (Hyde *et al.* 2020).

Based on morphology and molecular studies, *Amphisphaeria yunnanensis* and *Lepteutypa qujingensis sp. nov.* are introduced with descriptions, illustrations and morphological comparisons.

Materials and methods

Sample collection, isolation and morphological studies

Fresh materials were collected from a broadleaf evergreen forest area in Qujing, Yunnan Province, China (1618 m elevation) in May 2019. Specimens were placed in paper bags and carried to the laboratory. Ascomata were observed through a stereo microscope (SZX16, Olympus). Hand sectioning was carried out for observing internal characters. Microscopic characters were examined using a Nikon H5505 compound microscope. Sections were mounted in sterile water and measurements were recorded. Melzer's reagent was used to observe apical apparatus of asci. Average values were calculated for all the measurements and presented as ((minimum-maximum) $\overline{x}$ = average, n = number of observations). Photomicrography was conducted using a Canon EOS70D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work software and images used in figures were processed with Adobe Photoshop CS6 software (Adobe Systems, USA).

Single spore isolation was done according to Chomnunti *et al.* (2014) and germinating spores were transferred to potato dextrose agar (PDA). The pure cultures were incubated at 20–25 °C for 4 weeks. The type specimens were deposited in the Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica (HKAS), Chinese Academy of Science, Kunming and Chinese Academy of Science Herbarium (HMAS), Beijing, China. Ex-type cultures were deposited in the Kunming Institute of Botany Culture Collection (KUMCC), Chinese Academy of Science, Kunming and China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. Facesoffungi and Index Fungorum numbers were provided as outlined in Jayasiri *et al.* (2015) and Index Fungorum (http: www.indexfungorum.org) respectively. The detailed host, substrate and geographical locations of accepted species in both *Amphisphaeria* and *Lepteutypa* are provided in Tables 3 and 4.

DNA extraction and PCR amplification

Fungal isolates were grown on PDA for 3–4 weeks at 25 °C and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium scraped from the edges of the growing cultures (Wu *et al.* 2001). DNA extraction was followed by using the EZgne™ fungal gDNA kit (BIOMIGA, Hangzhou city, Zhejiang Province, China), according to the

manufacturer's protocol. DNA extracts were stored at –4 °C for use in regular work and duplicated at –20 °C for long term storage.

DNA sequence data was obtained from the partial sequences of two ribosomal coding genes including internal transcribed spacer region (ITS: ITS1-5.8S-ITS2) and 28S large subunit rDNA (LSU). ITS was amplified using primers ITS5 and ITS4 (White *et al.* 1990). LSU was amplified using primers LR0R and LR5 (Vilgalys & Hester 1990). Polymerase chain reaction (PCR) was carried out in a volume of 25 µl which contained 9.5 µL of ddH_2O, 12.5 µL of 2X PCR Master Mix (2x Bench Top™ Taq Master Mix, BIOMIGA, China), 1 µL of DNA template and 1 µL of forward and reverse primers (10 µM each) in each reaction. PCR thermal cycle program for all gene amplifications were as follows: initialization of 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s and elongation at 72 °C for 90 s, and final extension at 72 °C for 10 min. Purification and sequencing of PCR products were done by Sangon Biotech, Shanghai, China.

TABLE 1. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers.

Species	Culture collection/ Specimen number	LSU	ITS	Reference
A. acericola	MFLU 16–2479	MK640424	MK640423	Senanayake *et al.* 2019
A. flava	MFLUCC 18–0361*	MH971234	MH971224	Samarakoon *et al.* 2019
A. mangrovei	NFCCI–4247*	MG844275	MG844283	Phookamsak *et al.* 2019
A. sorbi	MFLUCC 13–0721*	KP744475	KR092797	Liu *et al.* 2015
A. thailandica	MFLU 18–0794*	MH971235	MH971225	Samarakoon *et al.* 2019
A. umbrina	HKUCC 994	AF452029	AF009805	Jeewon *et al.* 2003
A. yunnanensis	**KUMCC 19–0188***	**MN556306**	**MN477177**	**This study**
A. yunnanensis	**KUMCC 19–0189**	**MN550992**	**MN550997**	**This study**
Amphisphaeria acericola	MFLUCC14–0842*	MF614131	MF614128	Senanayake *et al.* 2019
Beltrania pseudorhombica	CBS 138003*	KJ869215	KJ869158	Crous *et al.* 2014
Beltraniella endiandrae	CBS 137976*	KJ869185	KJ869128	Crous *et al.* 2014
Beltraniopsis neolitseae	CBS 137974*	KJ869183	KJ869126	Crous *et al.* 2014
Lepteutypa aquatica	MFLUCC 14–0045*	MK835805	MK828607	Luo *et al.* 2019
L. fuckelii	CBS140409*	KT949902	KT949902	Jaklitsch *et al.* 2016
L. fuckelii	WU 33555	KT949903	KT949903	Jaklitsch *et al.* 2016
L. qujingensis	**KUMCC 19–0187***	**MN556316**	**MN477033**	**This study**
L. qujingensis	**KUMCC 19–0186**	**MN707567**	**MN707568**	**This study**
L. sambuci	CBS 131707*	KT949904	KT949904	Jaklitsch *et al.* 2016
L. sambuci	WU 33558	KT949906	KT949906	Jaklitsch *et al.* 2016
L. sambuci	WU 33558	KT949905	KT949905	Jaklitsch *et al.* 2016
L. uniseptata	HKUCC 6349*	DQ810219	N/A	Bahl *et al.* 2004

Types strains are indicated with (*) and newly generated sequences in this study are in bold. Unavailable sequence is indicated by "N/A". Abbreviations: ***CBS***—Centra albureau voor Schimmel cultures, Utrecht, The Netherlands; ***HKUCC***—University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; ***KUMCC***—Kunming Institute of Botany Culture Collection, Chinese Academy of Science, Kunming, China; ***MFLUCC***—Mae Fah Luang University Herbarium, Chiang Rai, Thailand; ***MFLUCC—***Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, ***NFCCI—***National fungal culture collection of India; ***WU***—Herbarium of the Institute of Botany, University of Vienna, Austria.

Molecular phylogenetic analyses

The sequence data generated in this study were analyzed with closely related taxa retrieved from GenBank (Table 1) based on BLASTn searches (https://www.ncbi.nlm.nih.gov) and recently published data (Samarakoon *et al.* 2019). Phylogenetic analyses were constructed based on ITS and LSU sequence data. The single gene alignments were automatically generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh *et al.* 2017), and were improved manually when necessary in BioEdit v. 7.0.5.2 (Hall 1999). ITS and LSU alignments were used to perform model test in MrModeltest 2.3 to estimate the best-fit evolutionary model under the Akaike information criterion (AIC) (Nylander 2004). Ambiguous regions were excluded from the analyses and gaps were treated as missing data.

Maximum likelihood analyses (ML) were performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2012) single and combined alignments. The optimal ML trees were obtained with 1,000 separate runs under the GTR + I + GAMMA substitution model resulting from model tests. Bayesian inference was performed using MrBayes on XSEDE tool in CIPRES (Larget & Simon 1999, Huelsenbeck & Ronquist 2001, Ronquist *et al.* 2012). Maximum-parsimony (MP) analysis was carried by using PAUP v. 4.0b10 (Swofford 2002). Each MP analyses were carried out with the heuristic search option and 1000 replicates. The Kishino-Hasegawa tests were performed to determine whether trees were significantly different (Kishino & Hasegawa 1989). Descriptive tree statistics for parsimony such as; the tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were documented. Posterior probabilities (PP) were obtained from Markov Chain Monte Carlo Sampling (BMCMC) (Rannala & Yang 1996, Ronquist *et al.* 2012) when the average standard deviation of split frequencies fell below 0.01. Markov Chain Monte Carlo (MCMC) chains were run from random trees for 1,000,000 generations and sampled every 100th generations with the burning value of 25%. The remaining trees were used to calculate PP values. All trees were visualized in FigTree v1.4.0 (Rambaut 2012) and the final layout was done with Microsoft PowerPoint. The final alignment and tree were registered in TreeBASE under the submission ID. 25669 (http://www.treebase.org/).

Results

Phylogenetic analyses

The combined LSU-ITS matrix consisted of 18 strains of Amphisphaeriaceae and three outgroup taxa in Beltraniaceae. The alignment contained 1358 characters (LSU: 1–836, ITS: 837–1358) including alignment gaps. The MP analysis resulted a single most parsimonious tree (TL = 435, CI = 0.674, RI = 0.701, CR = 0.472, HI = 0.326). The best scoring RAxML tree was selected to represent the relationships among taxa with a final likelihood value of –4231.272884 (Figure 1). The matrix had distinct alignment patterns with 7.2% of undetermined characters or gaps. Estimated base frequencies were as A = 0.256306, C = 0.213596, G = 0.266979, T = 0.263118; and substitution rates as AC = 0.633815, AG = 2.906967, AT = 1.264086, CG = 0.417930, CT = 5.263072, GT = 1.000000.

Amphisphaeria and *Lepteutypa* separate into two distinct clades with high statistical support from each analysis (100% MP, 100% ML, 1.00 PP). KUMCC 19–0188 and KUMCC 19–0189 strains cluster in *Amphisphaeria* (100% MP, 100% ML, 1.00 PP) which is close affinity to *A. thailandica* (MFLU 18–0794). KUMCC 19–0186 and KUMCC 19–0187 strains are sister to *L. fuckelii* with moderate statistical supports (79% MP, 84% ML, 0.99 PP). Phylogenetic analyses showed poor statistical supports among *Amphisphaeria* species while considerably strong for *Lepteutypa* species.

Taxonomy

Amphisphaeria yunnanensis L.S. Dissan., J.C. Kang & K.D. Hyde, ***sp. nov.*** (FIGURE 2)

Index Fungorum number: IF556876, *Facesoffungi number*: FoF 06505

Etymology:—The specific epithet *yunnanensis* refers to the province in which the fungus was collected

Holotype:—HMAS 290476

Saprobic on a dead branch. **Sexual morph** *Ascomata* 320–385 *μm* high × 380–450 *μm* diam. ($\bar{x}$ = 352.5 × 415 *μm*, n = 8), immersed, visible as black spots, solitary, scattered, globose to sub-globose, dark reddish brown, papillate. *Ostiole* 130−135 *μm* high × 28–32 *μm* diam. ($\bar{x}$ = 132.5 × 30 *μm*, n = 6). *Peridium* 8–18 *μm* ($\bar{x}$ = 13 *μm*, n = 10), comprising an inner layer of hyaline cells of *textura angularis*, and an outer layer of brown cells of *textura angularis*. *Paraphyses* 2–4.5 *μm* wide ($\bar{x}$ = 3.2 *μm*, n = 10), hyaline, few, longer than asci, cellular, constricted septate, guttulate, embedded in a gelatinous matrix, un-branched. *Asci* 78–93 × 6–9 *μm* ($\bar{x}$ = 85.5 × 7.5 *μm*, n = 20), 8-spored, unitunicate, cylindrical, with short pedicel, apically rounded, with a J- apical ring. *Ascospores* 12–15 × 4–6 *μm* ($\bar{x}$ = 13.5 × 5 *μm*, n = 30), overlapping uniseriate, fusiform, guttulate, hyaline when young, brown at maturity, uniseptate, constricted at the septum, smooth-walled. **Asexual morph** Undetermined.

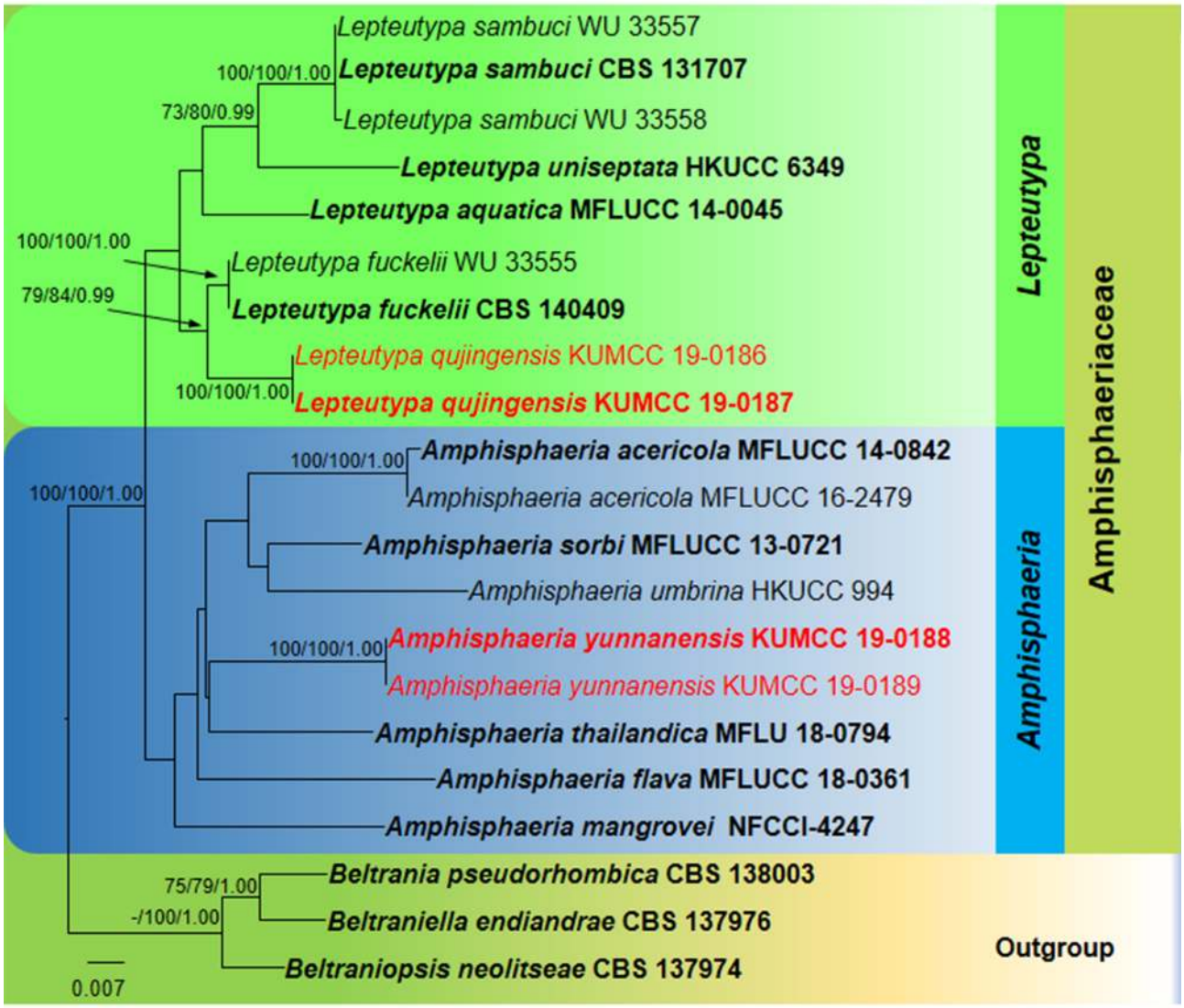

FIGURE 1. Phylogram generated from maximum likelihood (RAxML) based on LSU-ITS matrix. MP and ML bootstrap supports (≥70%) and Bayesian posterior probability (≥ 0.95) are indicated as MP/ML/BYPP. The tree is rooted to *Beltraniella endiandrae* (CBS 137976), *Beltraniopsis neolitseae* (CBS 137974) and *Beltrania pseudorhombica* (CBS 138003). Type strains are in bold and the newly generated strains are in red.

Culture characteristics:—Colonies on PDA, reaching 21.5 mm diam., after 2 weeks at 20–25 °C, medium dense, circular to slightly irregular with uneven margin, slightly raised and cottony surface, colony from above: white to pale grey at the margin, greenish-grey at the center; from below: yellowish white at the margin, yellow to brown at the center; mycelium greenish-grey.

Material examined:—CHINA, Yunnan province, Qujing (24.668703°N, 104.24653°E), on a dead branch of an unknown host, 06 May 2019, L.S. Dissanayake, DW1137−048 (HMAS 290476, **holotype**; HKAS 107066, **isotype**), ex-type living culture KUMCC 19−0188, CGMCC, additional materials DW1137−049 (HAMS 290477, HKAS 107067), living culture KUMCC 19−0189.

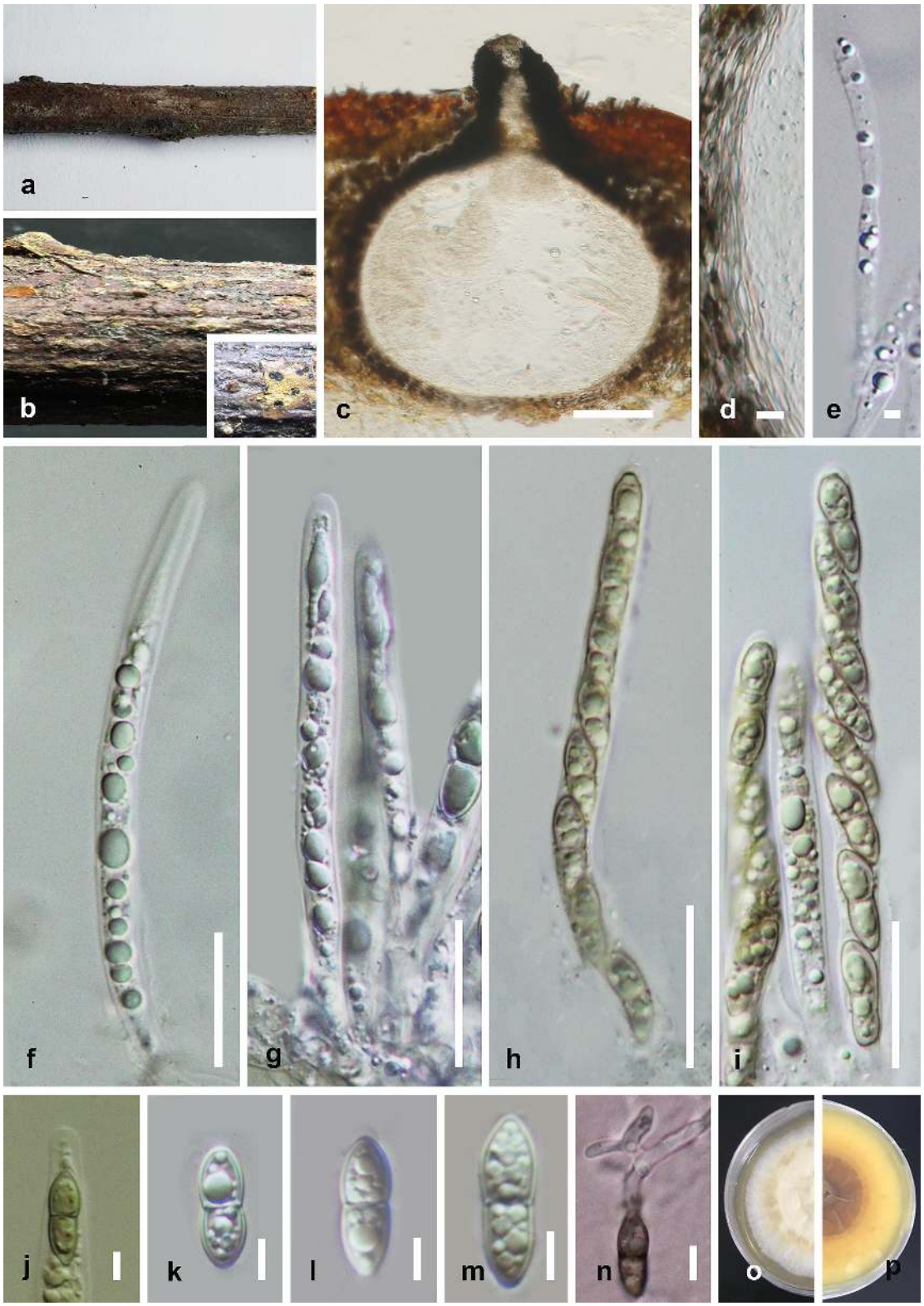

FIGURE 2. ***Amphisphaeria yunnanensis*** (HMAS 290476). a, b. Ascomata on the substrate. c. Vertical section of ascoma. d. Peridium. e. Paraphyses. f–i. Asci. j. J- Apical apparatus. k–m. Ascospores. n. Germinating ascospore. Culture on PDA from, o. above, p. below after 6 weeks. Scale bars: c = 100 *μm*, f–i = 20 *μm*, d,e, j–n = 5 *μm*.

Known distribution:—Yunnan Province, China

Notes:—Both of our specimens (HMAS 290476 and HAMS 290477) share similar characters typical of *Amphisphaeria* species in having immersed ascomata, 8-spored, unitunicate asci and overlapping uniseriate, brown, uniseptate ascospores. *Amphisphaeria yunnanensis* is morphologically similar to *A. bertiana* Fairm., *A. paedida* (Berk. & Broome), Sacc. and *A. vibratilis* (Fuckel) E. Mull in having J- apical rings. However, *Amphisphaeria bertiana*

has erumpent or superficial ascomata on a subiculum and *A. paedida* has superficial, coriaceous ascomata, while *A. yunnanensis* has immersed ascomata without a subiculum. *Amphisphaeria vibratilis* comprises with *textura intricata* cells in the peridium and larger ascospores (22.5–27.5 × 6–8.5 *μm*) with a mucilaginous sheath, while *A. yunnanensis* has compressed cells of *textura angularis* in the peridium and smaller ascospores (12–15 × 4–6 *μm*) without a sheath. *Amphisphaeria yunnanensis* has a close phylogenetic relationship with *A. thailandica*, but this is not supported in all formats of the analyses. However, *A. yunnanensis* differs from *A. thailandica* in having globose to sub-globose, dark reddish brown ascomata, long and narrow ostiole, fusiform, multi-guttulate ascospores. Moreover, *A. yunnanensis* differs from other remaining *Amphisphaeria* species in having narrow and long ostioles and small, fusiform, multi-guttulate ascospores. Significant characteristics among similar taxa are given TABLE 2. Based on phylogenetic evidences and morphological differences, we introduce our new collection as a new species as *A. yunnanensis*.

Lepteutypa qujingensis L.S. Dissan., J.C. Kang & K.D. Hyde, ***sp. nov.*** (FIGURE 3)

Index Fungorum number: IF556877, *Facesoffungi number*: FoF 06506
Etymology:—The specific epithet *qujingensis* refers to the city in which the fungus was collected
Holotype:—HMAS 290478

Saprobic on a dead branch. **Sexual morph** *Ascomata* 161–222 *μm* high × 525–550 *μm* diam. ($\overline{x}$ = 191.5 × 537.5 *μm*, n = 10), immersed, visible as minute dark black spots, flat on the host surface, solitary, scattered, sub-globose and brown. *Peridium* 10–25 *μm* ($\overline{x}$ = 17.5 *μm*, n =10), comprising an inner layer of hyaline cells of *textura angularis*, the outer layer of reddish brown cells of *textura angularis*. *Paraphyses* 6.5–12 *μm* wide ($\overline{x}$ = 9.3 *μm*, n = 15), hyaline, few, longer than asci, cellular, constricted at septum, guttulate, embedded in a gelatinous matrix. *Asci* 100–140 × 4.5–8.5 *μm* ($\overline{x}$ = 120 × 6.5 *μm*, n = 20), 8-spored, unitunicate, cylindrical, with long pedicel, apically rounded, with a J+ apical ring. *Ascospores* 19–26 × 4–6 *μm* ($\overline{x}$ = 22.5 × 5 *μm*, n =35), overlapping uniseriate, straight to slightly curved, fusiform, multi-guttulate, hyaline turning light brown when mature, 5–6-septate, smooth-walled **Asexual morph** Undetermined.

Culture characteristics:—Colonies on PDA, reaching 15–20 mm diam., after 2 weeks at 20–25°C, circular, flat, smooth surface, entire edge, slightly wooly, smooth margin, with concentric rings of wooly from above: ash white at the center; from below: light brown at the margin, dark brown at the center; mycelium ash white.

Material examined:—CHINA, Yunnan Province, Qujing (24.668703°N, 104.24653°E) on recently dead branch of an unknown host, 06 May 2019, L.S. Dissanayake, DW1137–045 (HMAS 290478, **holotype**; HKAS 107065, **isotype**), ex-type living culture, KUMCC 19–0187, CGMCC, additional material DW1137–046 (HAMS 290471), living culture KUMCC 19–0186. Additional sequence *rpb2*: MN729566.

Known Distribution:—Yunnan Province, China.

Notes:—Both of our specimens (HAMS 290478 and HAMS 290471) share characteristics typical to *Lepteutypa* in having long, cylindrical asci with J+ apical ring and uniseriate, multiseptate, brown ascospores. *Lepteutypa qujingensis* differs from other *Lepteutypa* species by having thin asci and smaller ascospores (19–26 × 4.3–6.3 *μm*). *Lepteutypa qujingensis* is similar in morphology to *L. fuckelii* in having immersed ascomata, J+ apical ring and multiguttulate, hyaline to brown ascospores. However, *L. fuckelii* has ascomata surrounded by grey clypeus and 4-septate, yellowish brown ascospores with a sheath, while *L. qujingensis* has 5–6-septate, brown ascospores without a sheath. Phylogenetic analyses show that *L. qujingensis* is sister to *L. fuckelii* Petr. (79% MP, 84% ML, 0.99 PP, Fig. 1). Significant characteristics among similar taxa are given in TABLE 3. Based on phylogenetic evidences and morphological differences, we introduce *L. qujingensis* as a new species.

Discussion

We introduce two novel species of Amphisphaeriaceae increasing the total number of *Amphisphaeria* species to 19 and *Lepteutypa* species to 16. Both species are recorded from dead twigs of dicotyledonous plants in Yunnan, China. Even though, *Amphisphaeria* is mostly found from dicotyledonous hosts in temperate regions, several studies introduce species from tropical and monocotyledonous hosts (Hyde *et al.* 1996, Wang 2004, Phookamsak *et al.* 2019, Samarakoon *et al.* 2019).

TABLE 2. Morphological comparisons of accepted *Amphisphaeria* species

Species	Seq. data	Known distribution	Host and substrate	Morphology (*μm*)			References
				Ascomata	**Asci**	**Ascospores**	
Amphisphaeria acericola	A	Italy, Forlì-Cesena	On branch of *Acer campestre* (Sapindaceae)	Immersed, 350 × 275	100–130 × 8.5–11, J+, bilobed pedicellate	16–24 × 6–8, overlapping uniseriate, ellipsoidal to oval	Senanayake *et al.* (2019)
A. bertiana	N/A	USA, New York	In cavities at the end of a rotting log	Seated on a subiculum, erumpent/ superficial, 350–500 diam.	110–145 × 5–6, J- apical ring, very long filiform stipe,	10.5–12.5 × 4–5, 2-cells, smooth wall	Fairm (1905) Wang *et al.* (2004)
A. depressa	N/A	USA, Hawaii, Kaihea	*Cassia bicapsularis* (Leguminosae)	Poorly developed clypeus, immersed beneath, 220–360 × 200–310	84–110 × 8–9, J+ discoid, sub apical ring	16–19 × 6–7, 2-cells, distoseptate, not or slightly constricted at the septum	Petrak (1953) Wang *et al.* (2004)
A. fallax	N/A	Austria, Weibkirchen	*Quercus robur* (Fagaceae)	Developing under a clypeus semi immersed to erumpent, 400–480 × 230–260	170–220 × 12–18, J+ discoid, sub apical ring	20–26 × 8–12, 2-cells, strongly constricted at the septum	De not (1865),Wang *et al.* (2004)
A. flava	A	Thailand, Chiang Mai, Chang Wat	On a recently dead branch	Immersed, black spots, surrounded by a pale-yellow halo on the surface 225–320 × 355–470	125–175 × 6.5–14.5, J+ discoid apical apparatus	13–16 × 5–7, rarely overlapping, hyaline to light brown	Samarakoon *et al.* (2019)
A. gaubae	N/A	Australia, Jervis Bay	Dead leaves of *Lambertia formosa* (Proteaceae)	Immersed beneath, blackened clypei, 450–520 × 420–500	100–140 × 10–11, J+ sub apical ring narrow wedge shape,	12.5–19 × 5.5–7.5, 2- cells, curve and pointed ends	Wang *et al.* (2004)
A. lusitanica	N/A	Portugal, Figueira da Foz	*Arundo donax* (Gramineae)	Immersed beneath a clypeus, 550–620 × 380–420	J+ sub apical ring wedge-shaped,	20–28 × 8–10, 2-cells, thin mucilaginous sheath	Wang *et al.*(2004)
A. mangorvei	A	India, Tamil Nadu	On intertidal branches and twigs of *Suaeda monoica*	Immersed to erumpent, 140 × 150	80–130 × 9.5–10, J-, apical ring	12–130 × 9 5–10, lacking a mucilaginous sheath	Phookamsak *et al.* (2019)
A. multipunctata	N/A	New Zealand, Bay of Plenty	*Ctinidia deliciosa* (Actinidiaceae)	Immersed beneath blackened clypei, 320–420 × 220–350	125–165 × 7.5–10, J+ discoid, sub apical ring	15–20 × 5–7, verruculose, rounded ends,	Petr *et al.*(1923) Wang *et al.*(2004)
A. paedida	N/A	Germany, Konigstein	Bark of wood	Superficial sub globose Coriaceous, 350–450	140–160 × 8–9, J- apical apparatus	16–18 × 5–6, 2- cell, guttules in each cell	Sacc *et al.*(1882) Wang *et al.*(2004)

.....continued on the next page

TABLE 2. (Continued)

Species	Seq. data	Known distribution	Host and substrate	Morphology (*µm*)			References
				Ascomata	**Asci**	**Ascospores**	
A. pseudoumbrina	N/A	Italy	On bark of *Acer campestre* (Aceraceae)	Immersed under a clypeus, 480–600 × 230–250	100–125 × 7.5–8.5, J+ discoid, sub apical ring	14–18 × 6–7.5, 2-cells, rugose-walled	Saccardo *et al.* (1873) Wang *et al.* (2004)
A. seriata	N/A	USA, Texas,	On leaf of *Nolina sp.* (Agavaceae)	Immersed, 320–380 × 300–360	112.5–150 × 11–12.5, J+ discoid, sub apical ring	15.5–20 × 6–8, 2-cells, deeply pigmented at septum, mucilaginous sheath	Barr *et al.* (1996) Wang *et al.*(2004)
A. sorbi	A	Italy, Trento	On branch of *Sorbus aucuparia* L. (Rosaceae)	Immersed to erumpent, 450–505 × 350–405	125–170 × 9–13, J- apical apparatus	16–24 × 6–8, rarely overlapping, ellipsoidal, thick mucilaginous sheath	Liu *et al.*(2015)
A. thailandica	A	Thailand, Phayao	On a recently dead branch	Immersed, flat or concave on the host surface, 210–265 × 410–470	95–120 × 9.5–16, J- apical apparatus	12.3–15 × 6.9–8.8, bi-guttulate, hyaline to light brown	Samarakoon *et al.* (2019)
A. umbrina	A	Italy, Flaventino	On trunk of *Ulmus sp.* (Ulmaceae)	Immersed, erumpent, 560–640 × 400–480	150–170 × 11–13, J+ discoid, sub apical ring,	18–22 × 6–8, 2-cell long ellipsoidal	De note (1863) Wang *et al.* (2004)
A. vibratilis	N/A	Canada, British Columbia	On the stem of *Prunus sp.* (Rosaceae)	Immersed, 720–960 × 400–500	137.5–187.5 × 10–15, J-, no visible ring in mature asci.	22.5–27.5 × 6–8.5, verrucose, surrounded by a mucilaginous sheath, deeply pigmented at septum,	Mull *et al.*(1962) Wang *et al.* (2004)
A. yunnanensis	A	China, Yunnan province	On recently dead branch attach to the host	Immersed, narrow papillate, 380–450 × 320–385, long and narrow ostiole (130.88 × 31.81)	78–93 × 6–9, J- apical ring	12–15 × 4–6, fusiform, uniseptate, constricted at the septum.	This study

- N/A= Not available, A= Available

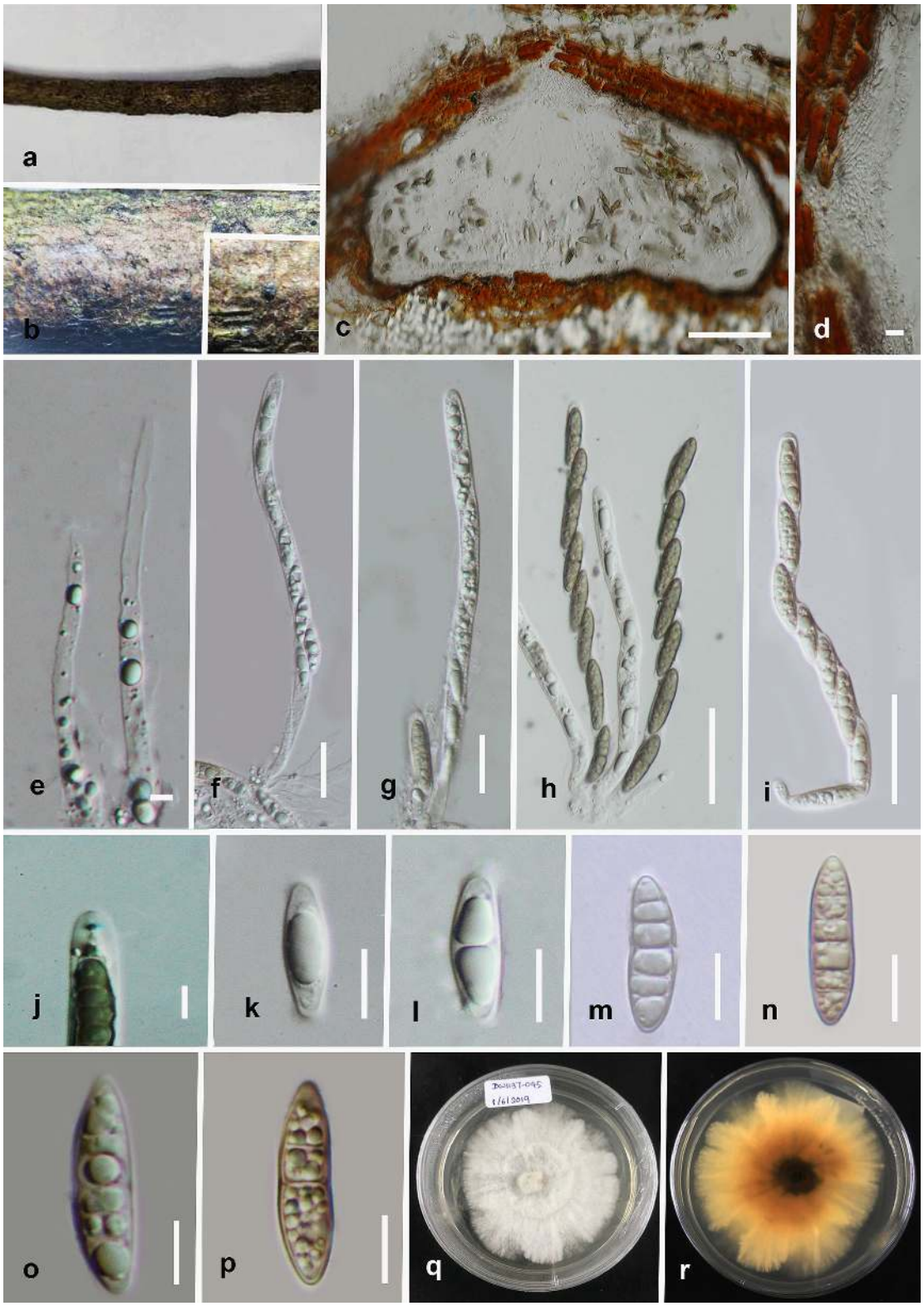

FIGURE 3. ***Lepteutypa qujingensis*** (HMAS 290478). a,b. Ascomata on the substrate. c. Vertical section of ascoma. d. Peridium. e. Paraphyses. f–i. Asci. j. Apical ring bluing in Melzer's reagent k–p. Ascospores. Culture on PDA from, q. above, r. below after 6 weeks. Scale bars: c = 100 *μm*, d, e = 5 *μm*, f–i = 20 *μm*, j = 5 *μm*, k–p = 10 *μm*.

There is very little molecular data available in public data bases for amphisphaeriaceous taxa. The phylogenetic analyses of this study are based on only ITS and LSU due to unavailability of other gene regions. In addition, there is a noticeable instability of the topologies among *Amphisphaeria* species in consecutive phylogenetic analyses (Phookamsak *et al.* 2019, Samarakoon *et al.* 2019, Senanayake *et al.* 2019). However, this may be resolved with future discoveries of species with molecular data.

TABLE 3. Morphological comparisons of accepted *Lepteutypa* species.

Species	Seq data	Known distribution	Host and substrate	Morphology (*µm*)			References
				Ascomata	**Asci**	**Ascospores**	
Lepteutypa alpestris	N/A	-	-	Irumpent, globose 440–550 × 15–20	100–110 × 7.5–15 apical ring shallow	20–27 × 5–7.5 3 septa Oblong/ellipsoid reddish brown narrow sheath	Barr (1993)
L. aquatica	A	Thailand	Submerged decaying wood in a freshwater	Immersed, sub-globose to depressed globose 250–320 × 300–330	126–138 × 8–10	15–17 × 5–7 oblong to reniform straight to slightly curved, guttulate, pale brown	Luo *et al.* (2019)
L. biseptata	N/A	East of Goulburn	-	Sub-epidermalia, globose 160–250 × 30–40	75–105 × 7–10	120–165 × 6–7 1–3 septa Oblong/ellipsoid mucous sheath	Petrak (1954)
L. cupressi	N/A	Australia	-	Glogose to suboblate 400 × 150	90–165 × 9–12 J J+ apical ring	14–23 × 6–9 3 septa Oblong to ellipsoidal Brown colour	Swart (1973)
L. fuckelii	A	Germany, Nordrhein Westfalen	On attached branches of *Tilia cordata*	Immersed surrounded by grey clype 450–570 × 200–400	111–155 × 10.5–13.3 J+ thin apical ring	17.5–22.8 × 6.5–8.0, 4 septa Oblong/ narrowly fusiform, hyaline to yellow-brwon narrow mucous sheath	Jaklitsch *et al.* (2016)
L. fusispora	N/A	Hawaiian Island	On *Wistaria sp.*	Globos, ellipsoidate 200–250 × 60–100	95–115 × 7–8.5	14–24 × 6.5–8	Petrak (1953)
L. hederae	N/A	Switzerland	On dead, corticated branches of *Hedera helix*	Immersed convex to pulvinate 350–700 × 250–600	Oblong, apex not containing a ring	22–29.2 × 9.2–12.2, ovoid to oblong,	Jaklitsch *et al.*(2016)
L. hexagonalis	N/A	Ecuador.	On dead trunk of *Pinanga sp.*	Immersed 700–800 × 600–700	180–210 × 7–10, J+ subepical ring,	27–32 × 6–7, 3-septate, fusiform	Goh *et al*, (1997)
L. qujingensis	A	Qujing city, Yunnan province, China	On recently dead branches attach to the host	Immersed, 161–222 × 525–550	103–140 × 4.8–8.3 J+ apical ring	19–26 × 4.3–6.3, 5–6 septa, thin wall	This study
L. sabalicola	N/A	Florida	On *Aralia spinosa*	Immersed in yellowish areas of substrate 260–495 × 130–275	44–65 × 7.5–10 J- apical ring	11–15.5 × 3.5–4.5 3-septate oblong/obovoid, reddish brown	Barr (1993)
L. sambuci	A	Germany, Yorkshire	On partly decorticated branches of *Sambucus nigra*	Erumpent, globose 400–800 × 250–500	175–228 × 12.3–16.8, J+ apical ring	24.5–31.3 × 9.0–11.2, rarely curved, 2–4 (-6) distoseptate, thick sheath	Jaklitsch *et al.*(2016)
L. ulmicola	N/A	Canada	On *Ulmus americana*	Immersed 325–455 × 300–350	108–150 × 9–11 J+ apical ring	17–21 × 8–9 3 septate ellipsoid/ oblong, light to dark brown	Barr (1993)

- N/A= Not available, A= Available

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