

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/301682286>

A new species of *Zasmidium* associated with sooty blotch and flyspeck

Article in *Phytotaxa* · April 2016

DOI: 10.11646/phytotaxa.258.2.9

CITATIONS

0

READS

44

7 authors, including:



Liu Gao

Northwest A & F University

13 PUBLICATIONS 18 CITATIONS

[SEE PROFILE](#)



Guangyu Sun

Northwest A & F University

59 PUBLICATIONS 281 CITATIONS

[SEE PROFILE](#)



A new species of *Zasmidium* associated with sooty blotch and flyspeck

WANYU ZHAO¹, YANMIN HOU¹, JINGKE BAI¹, WEI ZHANG¹, LIU GAO¹, RONG ZHANG^{1*}, MARK L. GLEASON² & GUANGYU SUN^{1*}

¹ State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi Province, China

² Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, United States of America

* Corresponding author

email: rongzh@nwsuaf.edu.cn (RZ); sgy@nwsuaf.edu.cn (GS)

During a survey to characterize the diversity of sooty blotch and flyspeck fungi in China, we obtained an isolate from a fungal colony exhibiting sooty blotch and flyspeck signs on the petiole of brown bollygum (*Litsea glutinosa*) in Guangxi, China. Based on phylogenetic analysis and morphological characterization, a new species, *Zasmidium litseae* is introduced. The new species is characterized by its swollen, straight or geniculate conidiogenous cells.

Key words: ectophyte; Mycosphaerellaceae; multigene; phylogenetic analysis

Introduction

Sooty blotch and flyspeck (SBFS) is a fungal disease occurring on a wide range of plant hosts. The disease blemishes the epicuticular wax layer, decreasing the aesthetic appearance and the sale value of fresh-market fruit. Although SBFS fungi have been studied for nearly 200 years, the identification of the causal agents remained uncertain until recent studies revealed that the complex comprised more than 80 species in more than 10 genera (Gleason *et al.* 2011). It is likely that many additional SBFS species remain to be discovered.

Zasmidium, one of the genera that includes SBFS species, was established by Fries in 1849. The type species of *Zasmidium*, *Z. cellare*, clusters in the Mycosphaerellaceae (Fries 1849, Arzanlou *et al.* 2007). Currently, 196 species of this genus have been documented in MycoBank with most species originating from plant hosts (Shivas *et al.* 2010, Crous *et al.* 2014, Quaadvlieg *et al.* 2014).

Previously, only one species of *Zasmidium*, *Z. angulare*, was reported as a causal agent of SBFS (Li *et al.* 2012). During a survey of the diversity of SBFS fungi in China, we obtained an isolate from a petiole of brown bollygum, *Litsea glutinosa* (Lour.) C.B. Rob., which was identified as a new species of *Zasmidium* based on phylogeny and morphology analysis. This is the first report of SBFS on *Litsea glutinosa*.

Material & Methods

Isolates

Petioles of *Litsea glutinosa* (Lour.) C.B. Rob. exhibiting SBFS colonies (Gleason *et al.* 2011) were collected in Beihai City, Guangxi, China, in October 2013. Thalli were transferred directly from colonies to potato dextrose agar (PDA) slants in a sterile environment and cultured at 25°C for 1 month in darkness (Sun *et al.* 2003). Mycelium was sub-cultured onto PDA plates in order to measure and observe fungal structures (Li *et al.* 2011). Microscopic examination was made after 7 days of incubation at 25°C and 30 measurements were obtained per structure. Colony descriptions were made after 2 weeks of growth on PDA plates at 25°C in the dark. Subsequently, pure cultures were stored in glycerol at –80°C.

Genomic DNA extraction, PCR, and sequencing

Genomic DNA was obtained according to the protocol of Li *et al.* (2011). The strain was amplified for two loci (ITS & LSU). The primer pairs for ITS and LSU were ITS1-F/ITS4 (White *et al.* 1990) and LSU1Fd/LR5 (Crous *et al.* 2009, Vilgalys & Hester 1990). Amplification reactions consisted of 1 unit of Taq polymerase (Thermo Scientific), 1× Taq buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.4 μM of the forward and reverse primers, and 2 μL of template DNA, and were made up to a total volume of 25 μL with sterile water. Reactions were performed on a Bio-Rad PCR Sypetiole S1000™ Thermal Cycler (Bio-Rad, USA). The amplification conditions were: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were sequenced by BGI (Beijing, China).

Sequence alignment

The sequences generated in this study were added to other sequences that were downloaded from GenBank (Table 1) and imported into BioEdit5.0.9.1 (Hall 1999). Preliminary alignments were performed using CLUSTAL-X (Thompson *et al.* 1997), then adjusted manually. Phylogenetic analysis of aligned DNA sequences was carried out with PAUP v.4.0b 10 for 32-bit Microsoft Windows (Swofford 2003). Heuristic searches were performed with 1,000 random sequence additions. Clade stability was assessed by 1,000 bootstrap replications. The sequences described in this study were deposited in GenBank; alignment and a representative tree were saved in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S17870>).

TABLE 1 Species, strain and GenBank accession numbers included in DNA analysis.

Species	Host or substrate	Collection number	ITS	LSU
<i>Teratosphaeria cryptica</i>	<i>Eucalyptus globulus</i>	CPC13842	GQ852799	GQ852689
<i>Zasmidium angulare</i>	<i>Malus domestica</i>	CBS 132094	JQ622088	JQ622096
<i>Z. aerohyalinosporum</i>	<i>Eucalyptus tectifica</i>	CBS 125011	KF901605	KF901930
<i>Z. anthuriicola</i>	<i>Anthurium</i> sp.	CBS 118742	FJ839626	KF251785
<i>Z. cellare</i>	wine cellar	CBS 146.36	EU041821	EU041878
<i>Z. citri</i>	<i>Acacia mangium</i>	CPC 10522	AY752145	GU214502
<i>Z. citri</i>	<i>Acacia mangium</i>	CBS 116366	KF901780	KF902138
<i>Z. lonicericola</i>	<i>Lonicera japonica</i>	CBS 125008	KF251283	KF902093
<i>Z. nabiacense</i>	<i>Eucalyptus</i> sp.	CPC 12748 = CBS125010	KF901607	KF901932
<i>Z. nocoxi</i>	twig debris	CBS 125009	GQ852842	KF902157
<i>Z. parkii</i>	<i>Eucalyptus grandis</i>	CBS 387.92	KF901785	KF902143
<i>Z. pseudoparkii</i>	<i>Eucalyptus grandis</i>	CBS 111049	KF901641	KF901976
<i>Z. pseudoparkii</i>	<i>Eucalyptus grandis</i>	CBS 110988	KF901640	KF901975
<i>Z. rothmanniae</i>	<i>Rothmannia engleriana</i>	CPC 22656 = CBS137983	KJ869135	KJ869192
<i>Z. scaevolicola</i>	<i>Scaevola taccada</i>	BRIP 52795	HM122240	HM122241
<i>Z. syzygii</i>	<i>Syzygium cordatum</i>	CPC 19792	KC005777	KC005798
<i>Z. xenoparkii</i>	<i>Eucalyptus grandis</i>	CBS 111185	KF901663	KF902002
<i>Z. litseae</i>	<i>Litsea glutinosa</i>	ZWY137	KT203781	KT203782

Results

DNA phylogeny

We constructed trees using ITS and LSU sequences by maximum parsimony methods (Fig. 1). The tree was rooted to *Teratosphaeria cryptica*. A total of 18 sequences were included in the alignment of ITS and LSU regions, which contained 1,148 characters. In the analysis, 865 characters were constant, 157 characters were parsimony informative, and 126 variable characters were parsimony uninformative. The first (TL=529, CI=0.7259, RI=0.7452, RC=0.5409, HI=0.2741) of the most parsimonious trees is presented (Fig. 1).

The ZWY137 strain clustered together with *Z. syzygii* forming a clade with a bootstrap value of 100%. Meanwhile the clade stayed close with the type species of *Zasmidium*, *Z. cellare*. This phylogram provided a preliminary guide for identification of the isolate used in the research.

Taxonomy

Zasmidium litseae G.Y. Sun & Wanyu Zhao, *sp. nov.* (Fig. 2) MycoBank MB 812971

Etymology—Named after its host genus.

Mycelium consisting of septate, branched, 1.06–3.16 µm diam. hyphae. *Conidiophores* lateral on mycelium, solitary, erect, unbranched, straight to somewhat flexuous, subcylindrical, 27.36–59.45 × 2.03–3.09 µm, 0–5-septate. *Conidiogenous cells* integrated, terminal, subcylindrical, unbranched, but apex frequently swollen, straight or once geniculate, with one to several conidiogenous loci, proliferating sympodially. *Conidia* ellipsoidal, subcylindrical or fusiform, straight to somewhat flexuous, apex obtusely rounded, base truncate, 0–2-septate, 4.20–16.72 × 1.61–2.76 µm.

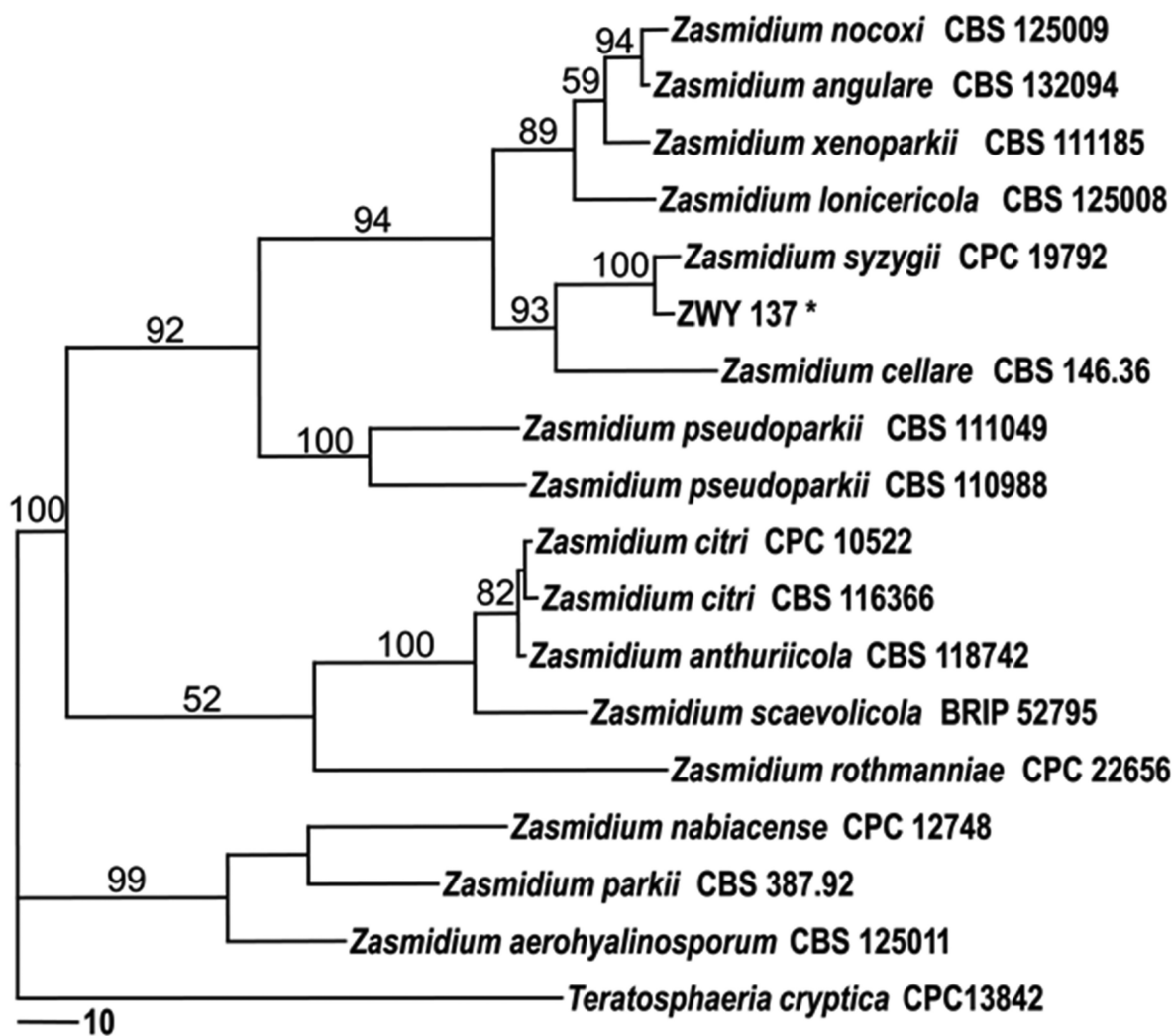


FIGURE 1. The first one of the parsimonious trees (TL=529, CI=0.7259, RI=0.7452, RC=0.5409, HI=0.2741). 18 sequences were comprised by the alignment of ITS and LSU region. The tree was rooted to *Teratosphaeria cryptica*.

Cultural characteristics—On PDA convex, margin entire, with moderate aerial mycelium, reaching 19 mm diam. after 2 weeks.

Mycelial type—Discrete speck.

Holotype—CHINA. Guangxi Province: Beihai City, 21°27'26"N, 109°3'37"E, on the petiole of *Litsea glutinosa* (Lour.) C.B. Rob, Oct. 2013, Wanyu Zhao and Liu Gao, HMAS 246718(=ZWY137) (dried culture), ex-type CGMCC3.17701 (=ZWY137).

Notes—*Zasmidium litseae* clusters close to *Z. syzygii* phylogenetically, but they are distinct in morphology. *Z. litseae* has shorter conidiophores and conidia, and its conidia have fewer septa. The conidiogenous cells of *Z. syzygii* taper apically towards a rounded or flattened apex, whereas the conidiogenous cells of *Z. litseae* are often swollen and geniculate towards the apex.

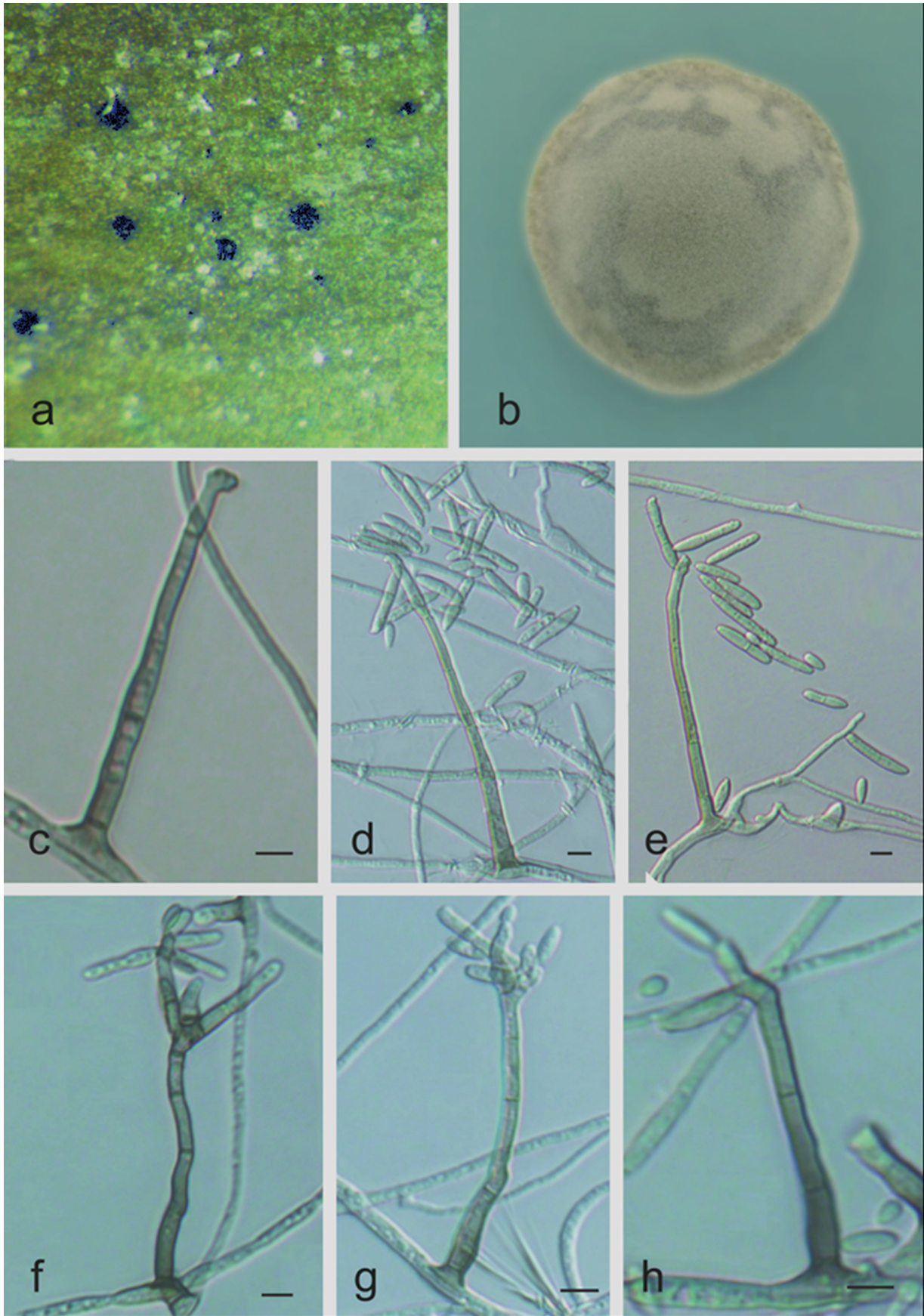


FIGURE 2. *Zasmidium litseae* (CGMCC3.17701). a. Signs on petiole of *Litsea glutinosa*. b. Colony on PDA. c–h. Conidiophores and conidia. Scale bars = 5 μ m

Acknowledgement

This work was supported by the National Natural Science Foundation of China (31371887, 31171797), the 111 Project from Education Ministry of China (B07049), CARS 28.

References

- Arzanlou, M., Groenewald, J.Z., Gams, W., Braun, U. & Crous, P.W. (2007) Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera. *Studies in Mycology* 58: 57–93.
<http://dx.doi.org/10.3114/sim.2007.58.03>
- Crous, P.W., Groenewald, J.Z., Summerell, B.A., Wingfield, B.D. & Wingfield, M.J. (2009) Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* 22: 38–48.
<http://dx.doi.org/10.3767/003158509X424333>
- Crous, P.W., Shivas, R.G., Quaedvlieg, W., van der Bank, M., Zhang, Y., Summerell, B.A., Summerell, B.A., Guarro, J., Wingfield, M.J., Wood, A.R., Alfenas, A.C., Braun, U., Cano-Lira, J.F., García, D., Marin-Felix, Y., Alvarado, P., Andrade, J.P., Armengol, J., Assefa, A., den Breeÿen, A., Camele, I., Cheewangkoon, R., De Souza, J.T., Duong, T.A., Esteve-Raventós, F., Fournier, J., Frisullo, S., García-Jiménez, J., Gardiennet, A., Gené, J., Hernández-Restrepo, M., Hirooka, Y., Hospenthal, D.R., King, A., Lechat, C., Lombard, L., Mang, S.M., Marbach, P.A.S., Marincowitz, S., Marin-Felix, Y., Montañó-Mata, N.J., Moreno, G., Perez, C.A., Pérez Sierra, A.M., Robertson, J.L., Roux, J., Rubio, E., Schumacher, R.K., Stchigel, A.M., Sutton, D.A., Tan, Y.P., Thompson, E.H., Vanderlinde, E., Walker, A.K., Walker, D.M., Wickes, B.L., Wong, P.T.W. & Groenewald, J.Z. (2014) Fungal Planet description sheets: 214–280. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 32: 184–306.
<http://dx.doi.org/10.3767/003158514X682395>
- Fries, E.M. (1849) *Summa Vegetabilium Scandinaviae* 2. HolmiaeA., Bonnier, pp. 259–572.
- Gleason, M.L., Batzer, J.C., Sun, G.Y., Zhang, R., Díaz Arias, M.M., Sutton, T.B., Crous, P.W., Ivanović, M., McManus, P.S., Cooley, D.R., Mayr, U., Weber, R.W.S., Yoder, K.S., Del Ponte, E.M., Biggs, A.R. & Oertel, B. (2011) A new view of sooty blotch and flyspeck. *Plant Disease* 95: 368–383.
<http://dx.doi.org/http://dx.doi.org/10.1094/PDIS-08-10-0590>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
<http://dx.doi.org/10.3114/sim.2007.58.03>
- Li, H.Y., Sun, G.Y., Batzer, J.C., Crous, P.W., Groenewald, J.Z., Karakaya, A. & Gleason, M.L. (2011) *Scleroramularia* gen. nov. associated with sooty blotch and flyspeck of apple and pawpaw from the Northern Hemisphere. *Fungal Diversity* 46: 53–66.
<http://dx.doi.org/10.1007/s13225-010-0074-9>
- Li, H.Y., Sun, G.Y., Zhai, X.R., Batzer, J.C., Mayfield, D.A., Crous, P.W., Groenewald, J.Z. & Gleason, M.L. (2012) Dissoconiaceae associated with sooty blotch and flyspeck on fruits in China and the United States. *Persoonia* 28: 113–125.
<http://dx.doi.org/10.3767/003158512X651157>
- Quaedvlieg, W., Binder, M., Groenewald, J.Z., Summerell, B.A., Carnegie, A.J., Burgess, T.I. & Crous, P.W. (2014) Introducing the Consolidated Species Concept to resolve species in the *Teratosphaeriaceae*. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 33: 1–40.
<http://dx.doi.org/10.3767/003158514X681981>
- Shivas, R.G., McTaggart, A.R., Young, A.J. & Crous, P.W. (2010). *Zasmidium scaevolicola*. *Fungal Planet* 47, *Persoonia* 24: 132–133.
- Sun, G.Y., Zhang, R., Zhang, Z. & Zhang, M. (2003) Isolation of sooty blotch and flyspeck fungi from apple surface by picking up the thalli. *Acta Phytopathologica Sinica* 33: 479–480.
- Swofford, D.L. (2003) PAUP*: Phylogenetic analysis using parsimony (* and other methods) version 4. Sinauer, Sunderland.
- 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: 4238–4246.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfe, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols, a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.
<http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1>