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Two new species of *Spencermartinsia* (*Botryosphaeriaceae*, Botryosphaeriales) from China

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Abstract

Spencermartinsia is a genus in *Botryosphaeriaceae* characterized by brown, 1-septate ascospores with an apiculus at either end, and usually found on woody hosts. Conidia are two-celled and become brown and 1-septate prior to dehiscence from conidiogenous cells. Based on morphological characteristics and phylogeny of DNA sequences of the internal transcribed spacer (ITS) region and part of the translation elongation factor 1–α (*tef1-α*) gene, two new species, namely *Spencermartinsia alpina* and *S. yunnana* are described from Yunnan province in China.

Key words – Asia – Dothideomycetes – phylogeny – taxonomy

Introduction

Spencermartinsia A.J.L. Phillips, A. Alves & Crous was introduced as a separate genus from *Dothiorella* Sacc. based on its brown, 1-septate ascospores with an apiculus at either end, and was typified by *S. viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous (Phillips et al. 2008). Species of *Spencermartinsia* can be saprophytes, pathogens and endophytes of various woody plants. Recently, more species have been added to *Spencermartinsia*, i.e. *S. citricola* A.J.L. Phillips & Abdollahz., *S. mangiferae* Abdollahz., Javadi & A.J.L. Phillips and *S. plurivora* Abdollahz., Javadi & A.J.L. Phillips from Iran, New Zealand, Spain and South Africa (Abdollahzadeh et al. 2014) and *S. rosulata* F.J.J. Van der Walt, Slippers & G.J. Marais from South Africa (Slippers et al. 2014) and *S. westrale* W.M. Pitt, J.R. Úrbez-Torres & Trouillas from Australia (Pitt et al. 2013). Thus, currently the genus *Spencermartinsia* includes six species (Mycobank http://www.mycobank.org, April 2016).

In the course of an ongoing survey of *Botryosphaeriaceae* in China, two undescribed species of *Spencermartinsia* were obtained from woody plants. In this paper we name and describe these species, and resolve their phylogenetic position within *Spencermartinsia*.

Materials & Methods

Isolates and morphology

Samples were obtained from dead branches (2.7–3.1 cm thick) of *Camellia* sp., *Acer*

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buergerianum, *Ternstroemia gymnanthera*, *Poncirus trifoliate* and *Platycladus orientalis* in Yunnan province, China in December 2014. Fresh samples were air-dried at room temperature and studied with an Olympus SZ 61 dissecting microscope without prior incubation. Microscopic observations were made from material mounted in lactic acid. Photomicrographs were taken with a Nikon Coolpix 995 digital camera on a Leitz Orthoplan microscope. Measurements of conidia, paraphyses and conidiogenous cells were made from lactic acid mounts.

Isolations were made from pycnidia on dead or dying wood and transferred to malt extract agar (MEA), and subsequently transferred to synthetic nutrient-poor agar (SNA) with sterilized pine needles to induce sporulation. Isolates were kept at ambient temperatures (about 25–30 °C) in the dark to determine colony characteristics.

DNA extraction, PCR amplification and sequencing

DNA was extracted with CTAB plant genome DNA fast extraction kit (Aidlab Biotechnologies Co, Ltd, Beijing, China) from mycelium grown on MEA. The internal transcribed spacer of rDNA (ITS) was amplified and sequenced with primers ITS1 and ITS4 (White et al. 1990). The translation elongation factor-1a (*tef1-α*) was amplified and sequenced with primers EF1-688F and EF1-1251R (Alves et al. 2008). DNA amplification and sequencing followed the protocol of Zhang et al. (2009).

Sequence alignment and phylogenetic analysis

The combined ITS and *tef1-α* nucleotide sequences were used to infer the phylogenetic relationships among *S. alpina* and *S. yunnana* and other species of *Spencermartinsia* by maximum parsimony (MP) and bayesian analyses. Sequences generated were analyzed with other sequences obtained from GenBank (Table 1). A multiple alignment was done on MEGA 5 (Tamura et al. 2011) and prior to the phylogenetic analyses, leading or trailing gaps at the start and end were deleted. For bayesian analysis, the best-fit model of nucleotide evolution (GTR+I+G) was selected using the Akaike information criterion (AIC; Posada & Buckley 2004) in MrModeltest 2.3. The metropolis-coupled Markov Chain Monte Carlo (MCMCMC) approach was used to calculate posterior probabilities (Ronquist & Huelsenbeck 2003). A preliminary Bayesian inference (BI) analysis using MrBayes revealed that the Markov Chain Monte Carlo (MCMC; Huelsenbeck & Ronquist 2001) steady state was reached after less than 50,000 generations. A conservative burn-in of 500 trees was chosen and a full analysis of 5,000,000 generations was carried out with sampling every 100 generations. Maximum Parsimony (MP) analysis was conducted in PAUP v. 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search option with 1,000 random sequence additions and tree-bisection-reconnection (TBR) as the branch-swapping algorithm and gaps are treated as missing data. Maxtrees were setup to 50,000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI]) were calculated for trees generated under different optimality criteria. Trees were viewed in TREEVIEW (Page 1996). Nucleotide sequences generated in this paper were deposited in GenBank. Trees and alignments were deposited in TreeBase with study ID S19496.

Results

Phylogenetic analyses

Phylogenetic analysis of the ITS and *tef1-α* sequence data comprising 887 bp from rDNA revealed 130 parsimony-informative characters. The outgroup taxon was *Neofusicoccum luteum*. The heuristic search with random addition of taxa (1,000 replicates) generated a single most parsimonious tree of 214 steps (CI = 0.864, RI = 0.915, RC = 0.791, HI = 0.136). Bayesian analysis resulted in a tree with similar topology. The Bayesian tree (Fig. 1) was selected to explain systematic relationships within *Spencermartinsia* and revealed the new species introduced in this study, namely *Spencermartinsia alpina* and *S. yunnana*.

Table 1 Details of *Spencermartinsia* strains and other species considered in this study.

Species	Strain	Host	Origin	GenBank Accession no.	
				ITS	*tef1-α*
Dothiorella pretoriensis	CMW 36480	*Acacia karroo*	Pretoria, South Africa	JQ239405	JQ239392
	CMW 36481	*A. karroo*	Pretoria, South Africa	JQ239406	JQ239393
D. uruguayensis	UY 672	*Hexachlamis edulis*	Paysandu, Uruguay	EU080923	EU863180
S. alpina	CGMCC 3.18001	*Platycladus orientalis*	Yunnan, China	**KX499645**	**KX499651**
S. citricola	ICMP 16827	*Citrus sinensis*	New Zealand	EU673322	EU673289
	ICMP 16828	*C. sinensis*	New Zealand	EU673323	EU673290
S. mangiferae	IRAN 1545C	*Mangifera indica*	Hormozgan, Iran	KC898223	KC898206
	IRAN 1546C	*M. indica*	Hormozgan, Iran	KC898222	KC898205
	IRAN 1584C	*M. indica*	Hormozgan, Iran	KC898221	KC898204
S. plurivora	IRAN 1556C	*Citrus sp.*	Hormozgan, Iran	KC898226	KC898209
	IRAN 1538C	*C. sempervirens*	Hormozgan, Iran	KC898229	KC898212
	IRAN 1552C	*Casuarina equisetifolia*	Hormozgan, Iran	KC898228	KC898211
	IRAN 1553C	*Malus domestica*	Hormozgan, Iran	KC898227	KC898210
	IRAN 1557C	*Citrus sp.*	Hormozgan, Iran	KC898225	KC898208
	IRAN 1556C	*Prunus armeniaca*	Hormozgan, Iran	KC898230	KC898213
	CJA 257	*Eucalyptus* sp.	Hormozgan, Iran	KC898224	KC898207
	DAR 78869	*Vitis vinifera*	Eden Valley, Australia	EU603287	HM800507
	DAR 78872	*V. vinifera*	Adelaide Hills, Australia	EU603292	HM800510
S. rosulata	CBS 121760	*A. karroo*	Windhoek, Namibia	EU101290	EU101335
	CBS 121761	*A. mellifera*	Pretoria, South Africa	EU101293	EU101338
	CMW 25394	*A. karroo*	Northern Cape, South Africa	EU101318	EU101363
	CBS 121762	*A. mellifera*	Northern Cape, South Africa	EU101319	EU101364
	CMW 25396	*A. mellifera*	Northern Cape, South Africa	EU101320	EU101365
	CMW 25397	*A. tortillis*	Northern Cape, South Africa	EU101321	EU101366
	CMW 25398	*A. tortillis*	Northern Cape, South Africa	EU101322	EU101367
S. viticola	CBS 117009	*V. vinifera*	Vimbodi, Spain	AY905554	AY905559
	CBS 117010	*V. vinifera*	Sant Esteve Sesrovires,Spain	AY905558	AY905561
S. westrale	DAR 80529	*V. vinifera*	Upper Swan, Australia	HM009376	HM800511
	DAR 80530	*V. vinifera*	Upper Swan, Australia	HM009377	HM800512
	DAR 80531	*V. vinifera*	Upper Swan, Australia	HM009378	HM800513
S. yunnana	CGMCC 3.17999	*Camellia* sp.	Yunnan, China	**KX499643**	**KX499649**
	CGMCC 3.18000	*Camellia* sp.	Yunnan, China	**KX499644**	**KX499650**
	CGMCC 3.17998	*Acer buergerianum*	Yunnan, China	**KX499646**	**KX499652**
	CGMCC 3.17997	*Ternstroemia gymnanthera*	Yunnan, China	**KX499641**	**KX499647**
	CGMCC 3.17996	*Poncirus trifoliata*	Yunnan, China	**KX499642**	**KX499648**
Neofusicoccum luteum	CBS 110299	*V. vinifera*	Oeiras, Portugal	AY259091	AY573217
	CBS 110497	*V. vinifera*	Portugal	EU673311	EU673277

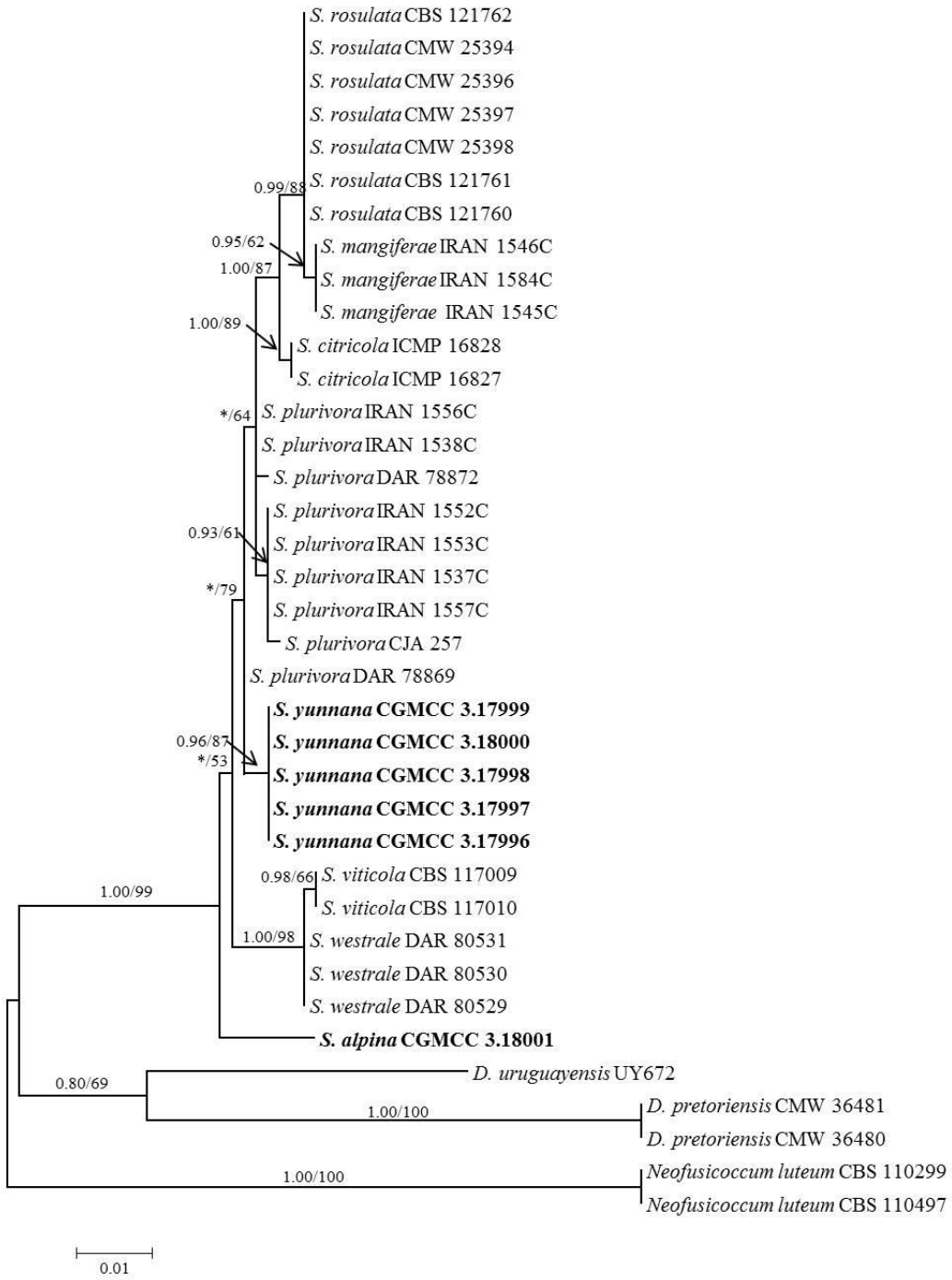

Fig. 1 – Phylogram of the Bayesian analysis based on combined ITS and *tef1-α* sequences with *Neofusicoccum luteum* species as outgroup. Numbers above branches indicate posterior probabilities/bootstrap values (* = value less than 0.90).

Taxonomy

Spencermartinsia alpina Y. Zhang ter., M. Zhang **sp. nov.** Fig. 2
MycoBank 817231

Etymology – Latin, *alpina*, referring to the Zixi mountain where the species was first found.

Conidiogenesis holoblasic, with conidiogenous cells cylindrical to fusiform and slightly utriform, hyaline, thin-walled, smooth, measuring (5.1–)6.3–10(–12.4) × 3–5.2 µm and giving rise to periclinal thickenings. *Conidia* initially hyaline, thin-walled, unicellular (non-septate) becoming thick-walled, dark brown and 1-septate prior to release from the conidiogenous cells, with rounded

apex or occasionally truncate base measuring (17.2–)19–20.4(–22.4) × (7.9–)8.4–9(–9.7) µm, with a mean length and width of 19.7 ± 1.5 × 8.7 ± 0.9 µm, and an average length to width ratio of 2.2 ± 0.2, slightly constricted at the septa when mature. *Conidial wall* thickness of 0.6–1.2 µm, with a mean thickness of 0.9 µm (n = 20).

Cultural characteristics – On MEA colonies initially were white with sparse aerial mycelium, and then become denser with white oppressed mycelia radiating outwards, forming small white cottony pycnidial primordia over the surface of the colony after 10 days; turning olivaceous gray gradually, reverse leaden gray.

Cardinal temperatures for growth – Between 5 °C and 35 °C with an optimum of 25 °C.

Habitat – *Platycladus orientalis*.

Specimens examined – CHINA, Yunnan province, Chuxiong city, Zixi mountain forest park (altitude: 2,000 m), from thick branch of dead young tree of *Platycladus orientalis*, 16 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254733, CGMCC 3.18001).

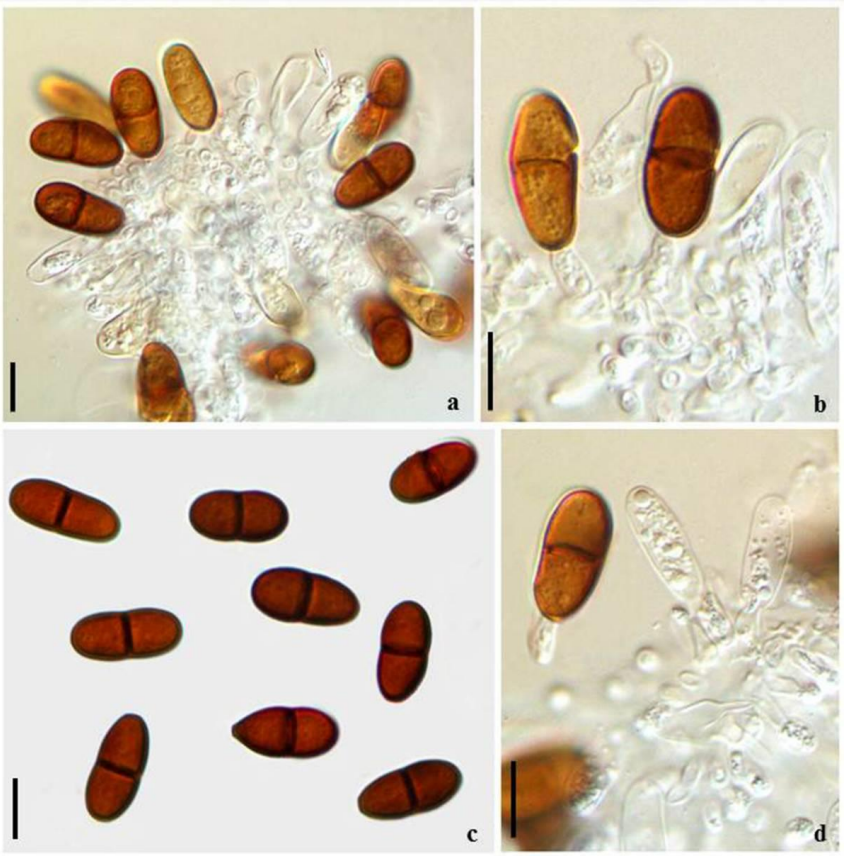

Fig. 2 – *Spencermartinsia alpina* (HMAS254733). a, b, d Hyaline immature and mature brown 1-septate conidia developing on conidiogenous cells. c Mature brown 1-septate conidia. – Bars = 10 µm.

Spencermartinsia yunnana Y. Zhang ter., M. Zhang **sp. nov.** Fig. 3
MycoBank 817233

Etymology – The epithet *yunnana* refers to Yunnan province, the place where it was first isolated.

Conidiogenesis holoblastic, with conidiogenous cells clavate to cylindrical and slightly utriform, hyaline, thin-walled, smooth, measuring (5.3–)6.5–10.2(–12.6) × 3.2–5.4 µm. *Conidia* ellipsoid, initially hyaline, thin-walled, non-septate becoming brown and 1-septate, externally smooth, occasionally slightly constricted at the septum with rounded apex and truncate base measuring (18.4–)19.6–21(–22.2) × (8.1–)8.6–9.2(–9.6) µm, with a mean length and width of 20.3 ± 1.5 × 8.9 ± 0.9 µm, and a mean length to width radio of 2.3 ± 0.2 (n = 30).

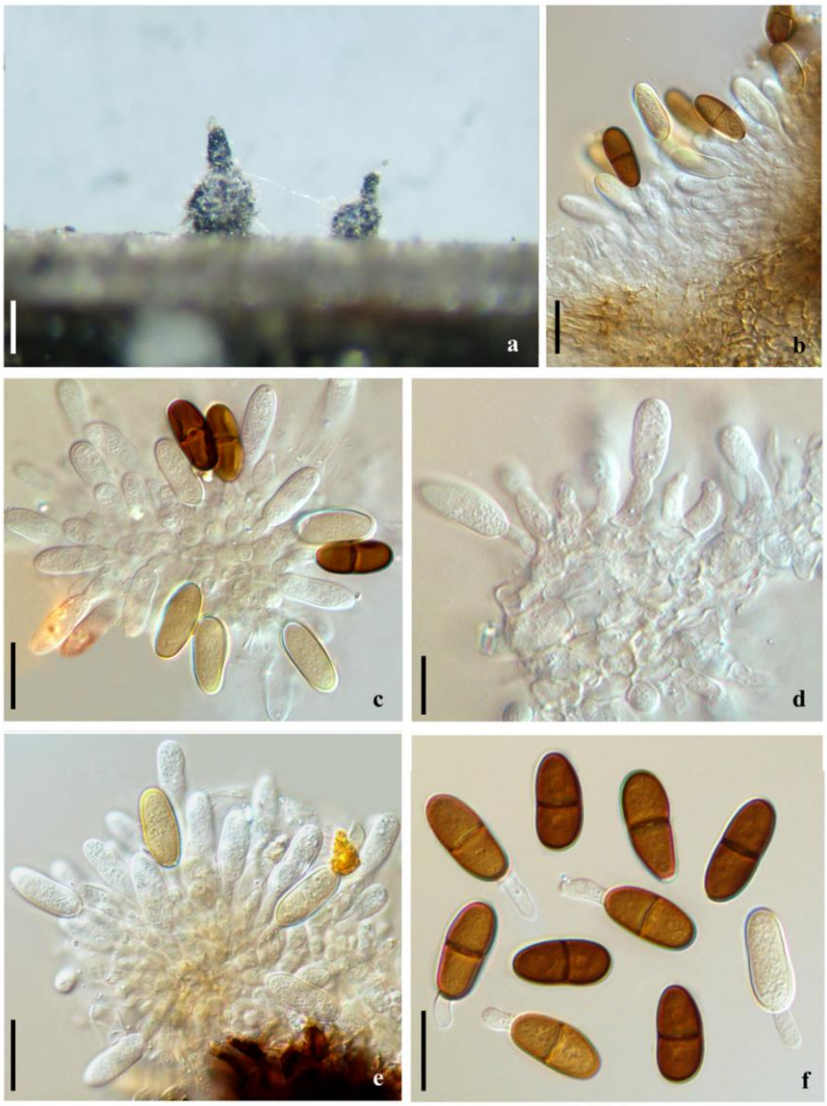

Fig. 3 – *Spencermartinsia yunnana* (a from CGMCC 3.17999, b–f from HMAS254734). a Conidiomata formed on poplar twigs in culture. b Brown 1-septate conidia attached to the conidiogenous cells. c Hyaline immature conidia attached to conidiogenous cells. d, e Immature aseptate conidia on conidiogenous cells. f Mature conidia. – Bars : a= 1 mm, b–f = 20 μm.

Cultural characteristics – On MEA colonies initially were white appressed mycelia radiating outwards, mycelium becoming more flourishing near the margin of the colony. In the center, with white sparse aerial mycelium within 10 days; turning dark slate-blue with olivaceous gray mycelium within 28 days, the reverse leaden gray.

Cardinal temperatures for growth – Between 5 °C and 35 °C with an optimum of 25 °C.

Habitat – *Camellia* sp.

Specimens examined – CHINA, Yunnan province, Chuxiong city, Zixi mountain forest park, from dead branch of *Camellia* sp., 16 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254734, CGMCC 3.17999).

Additional specimens examined – CHINA, Yunnan province, Chuxiong city, Zixi mountain forest park, from dead branch of *Camellia* sp., 16 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254735, CGMCC 3.18000); from dead branch of *Acer buergerianum*, 16 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254736, CGMCC 3.17998); Yunnan province, Kunming city, Kunming arboretum, on the dead twigs of the *Ternstroemia gymnanthera*, 15 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254738, CGMCC 3.17997); on the dead twigs of the *Poncirus trifoliate*, 15 Dec 2014, leg. W. He & J.R. Wu, det. Y. Zhang (HMAS254737, CGMCC 3.17996).

Discussion

Spencermartinsia is a cosmopolitan genus and *S. viticola*, the type species, has been reported from four different woody hosts (mostly *Vitis vinifera*) in China, South Africa, Spain, USA and Uruguay (Abdollahzadeh et al. 2014). In this study, two new species of *Spencermartinsia*, namely *S. alpina* and *S. yunnana*, were collected from Yunnan province, the subtropical area of China.

Only asexual states were observed on the specimens collected. Phylogenetic analyses based on combined ITS and *tef1-α* sequences indicated that *Spencermartinsia alpine* and *S. yunnana* form sibling clades with all other reported species respectively. Morphologically, the dark-brown, thick-walled (mean = 0.9 μm, n = 20) and slightly constricted conidia of *S. alpina* can be readily distinguished from all other reported species of *Spencermartinsia*. *Spencermartinsia yunnana* morphologically resembles *S. magniferae* and *S. viticola*, while the conidia of *S. yunnana* are more slender (mean L/W ratio: 2.3) than those of S. *magniferae* (mean L/W ratio: 2.1) and *S. viticola* (mean L/W ratio: 1.9 to 2.2) (Pitt et al. 2013, 2015). Apparently the sexual morph of *Spencermartinsia viticola* tends to occur with its asexual stage (Pitt et al. 2013, 2015). However, the sexual morph of *S. yunnana* was not seen in the present study.

Spencermartinsia yunnana was associated with a wide range of hosts including *Camellia* sp., *Ternstroemia gymnanthera* (Theaceae), *Acer buergerianum* (Sapindaceae) and *Poncirus trifoliate* (Rutaceae), and was collected from both Zixi mountain forest park (altitude, 2500 m) and Kunming arboretum (altitude, 1970 m), which may indicate that it is widespread in this region. A single isolate of *S. alpina* was obtained in this study from *Platycladus orientalis* (Cupressaceae). Its phylogenetic distinction from other species of *Spencermartinsia* supports its status as a new species.

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