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Aplosporella ginkgonis (Aplosporellaceae, Botryosphaeriales), a new species isolated from *Ginkgo biloba* in China

Du Z[1], Fan XL[1], Yang Q[1], Hyde KD[2] and Tian CM [1*]

[1] *The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, China*
[2] *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

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Abstract

Aplosporella ginkgonis sp. nov., is described from symptomatic branches of *Ginkgo biloba* in China based on morphological and molecular analysis. It is characterized by multiloculate conidiomata, with one to four ostioles, and aseptate, brown, ellipsoid to oblong conidia. Morphological and phylogenetic analyses of ITS, and tef1-α sequence data, support the position of the new species in *Aplosporella*, which forms a monophyletic lineage with strong support (MP/BI = 100/1). Thus, a new species is introduced in this paper to accommodate this taxon.

Key words – Ascomycetous fungi – Canker disease – Dothideomycetes – Systematics – Taxonomy

Introduction

Aplosporella (Aplosporellaceae) was introduced by Spegazzini (1880) and has frequently been incorrectly synonymized, especially under *Haplosporella* (Tilak & Rao 1964, Petrak & Sydow 1927, Tai 1979, Wei 1979). In addition, the introduction of most new species was based on host occurrence, whereas recent studies suggest that taxa in this genus are not host-specific (Fan et al. 2015, Ekanayaka et al. 2016, Dou et al. 2017). More than 337 epithets for *Aplosporella* are listed in Index Fungorum (April 2017) with an estimated 66 species in Kirk et al. (2008).

Synonymies and frequent name changes in botryosphaerialean taxa, including *Aplosporella*, have caused much confusion for plant pathologists and mycologists (Dissanayake et al. 2016). Thus a systematic revision of *Aplosporella* is needed, which takes into account host, morphology and phylogenetic analysis. Revisiting all old epithets in *Aplosporella* would be ideal but is beyond most expectations (Dayarathne et al. 2016). *Aplosporella* is circumscribed by multiloculate conidiomata with a single ostiole, and brown, aseptate conidia. Slippers et al. (2013) recognized the genus should be separate from Botryosphaeriaceae as a distinct family, which they named Aplosporellaceae, and confirmed that *Aplosporella* is well-resolved with species described from living culture. However, Liu et al. (2016), using evolutionary evidence, questioned whether this family was introduced unnecessarily and should be regarded as a genus. Slippers et al. (2013) found a consistent clustering between *Aplosporella* and another similar genus *Bagnisiella* Speg. and considered that *Bagnisiella* represents the sexual morph and should be reduced to synonymy with *Aplosporella* (Schoch et al. 2009, Slippers et al. 2013). Ekanayaka et al. (2016) introduced a new species named *Aplosporella thailandica* from Thailand with similarities to *Bagnisiella.* Fresh

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Corresponding Author: Chengming Tian – e-mail – chengmt@bjfu.edu.cn

collections of the latter genus are needed.

There are few reports of *Aplosporella* species from China, except for *Haplosporella* (currently *Aposporella*) species identified by host association and listed in Sylloge Fungorum Sinicorum and Identification of Fungus Handbook (Tai 1979, Wei 1979). Fan et al. (2015) isolated *A. javeedii* from five different hosts in China, which was previously regarded as an endophyte in South Africa (Jami et al. 2014). Subsequently, Dou et al. (2017) described *A. macropycnidia* from *Cerasus yedoensis* in China.

During studies on forest pathogenic fungi causing canker or dieback diseases in China, we unexpectedly isolated an *Aplosporella* species from *Ginkgo biloba* L. and *Morus alba* L. Following molecular and morphological study, we found this to be an undescribed species typical of *Aplosporella*. Therefore, in this paper we introduce *Aplosporella ginkgonis* sp. nov., with illustrations and compare it with other species in the genus.

Materials & Methods

Isolates

Two specimens were collected from infected branches or twigs of *Ginkgo biloba* and *Morus alba* from Gansu in China. Two strains of *Aplosporella* were obtained following the methods of Chomnunti et al. (2014) (Table 1). The suspension of conidia was inoculated from the conidiomata on to the surface of 1.8 % potato dextrose agar (PDA) in a Petri-dish, and incubated at 25 °C for up to 24 h. Single germinating conidia were aseptically transferred to fresh culture plates. Living cultures are deposited and now maintained at the China Forestry Culture Collection Center (CFCC) and Beijing Forestry University (BJFU) under strain numbers CFCC 50465 and CFCC 50466. Specimens are deposited in the Museum of the Beijing Forestry University (BJFC) under collection numbers BJFC-S1313 and BJFC-S1314.

DNA amplification, sequencing and phylogeny

Fungal mycelium grown on PDA was scraped from the agar and used for genomic DNA extraction using a CTAB method (Doyle & Doyle 1990). The ITS region was amplified using primers ITS1 and ITS4 (White et al. 1990). The tef-1α region was amplified using EF1-688F and EF1-1251R primers (Alves et al. 2008). DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China). DNA sequences generated from this study were deposited in GenBank (Table 1), and a data matrix were deposited in TreeBASE (www.treebase.org) as accession S20694. The multiple sequence alignment of isolates selected from recent studies and GenBank were generated with MAFFT v.7 (Katoh & Standley 2013).

Phylogenetic relationships were inferred by maximum parsimony (MP), and confirmed using MrBayes v.3.1.2 for Bayesian inference (BI) (Ronquist & Huelsenbeck 2003). An MP search was performed using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping. Maxtrees were set to 5,000, branches of zero length were collapsed and all equally parsimonious trees were saved. Descriptive tree statistics (Tree Length [TL], Consistency Index [CI], Retention Index [RI] and Rescaled Consistency [RC] were calculated. Bayesian inference (BI) analysis employing a Markov Chain Monte Carlo (MCMC) method were performed with MrBayes (Rannala & Yang 1996). A nucleotide substitution model was estimated by MrModeltest v.2.3 (Posada & Crandall 1998). For the analysis, two MCMC chains were run simultaneously, starting from random trees for 1,000,000 generations, and trees were sampled every 100th generation for a total of 10,000 trees. The first 25 % of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian posterior probabilities (BPP) were estimated in the remaining 7,500 trees. Trees were visualized with FigTree v.1.4.0 (http://tree.bio.ed.ac.uk software/figtree) and the layouts were prepared with Adobe Illustrator CS v.6.

Morphology

Characters of the fruiting bodies were observed on infected plant tissues and in culture. Macromorphological fungal structures were identified and studied with a Leica stereomicroscope (M205 FA), including size and arrangement of stromata, shape and size of ectostromatic disc and ostiole. Micromorphological observations with a Leica compound microscope (DM 2500) included the size and shape of conidiophores and conidia. More than 20 conidiomata were sectioned and 50 conidia were evaluated randomly for measurement. Cultural characteristics of strains incubated on PDA in the dark at 25 °C were recorded, including colony colour and conidiomata distribution. Taxonomic novelty was deposited in Faces of fungi numbers (FoF 02457) obtained as in Jayasiri et al. (2015).

Table 1 Strains of *Aplosporella* used in the molecular analyses in this study.

Species	Strain	Host	GenBank Assession Number	
			ITS	tef1-α
Aplosporella africana	CBS 121777	*Acacia mellifera*	EU101315	EU101360
Aplosporella africana	CBS 121779	*Acacia mellifera*	EU101317	EU101362
Aplosporella africana^{T}	CMW 25424	N/A	KF766196	EU101360
Aplosporella artocarpi^{T}	CPC 22791	*Artocarpus heterophyllus*	KM006450	KM006481
Aplosporella hesperidica	CBS 208.37	N/A	JX681069	N/A
Aplosporella javeedii	CFCC 50052	*Gleditsia sinensis*	KP208838	KP208844
Aplosporella javeedii	CFCC 50053	*Sophora japonica*	KP208839	KP208845
Aplosporella javeedii	CFCC 50054	*Juniperus chinensis*	KP208840	KP208846
Aplosporella javeedii^{T}	CFCC 89657	*Albizia julibrissin*	KM030579	KM030593
Aplosporella ginkgonis	CFCC 89660	*Morus alba*	KR045623	KP310847
***Aplosporella ginkgonis*^{T}**	CFCC 89661	*Ginkgo biloba*	KM030583	KM030597
Aplosporella macropycnidia^{T}	CGMCC3.17725	*Cerasus yedoensis*	KT343648	KX011176
Aplosporella macropycnidia	CGMCC3.17726	*Cerasus yedoensis*	KT343649	KX011177
Aplosporella macropycnidia	CGMCC3.17727	*Cerasus yedoensis*	KT343647	KX011175
Aplosporella papillata^{T}	CBS 121780	*Acacia tortillas*	EU101328	EU101373
Aplosporella papillata	CBS 121781	*Acacia erioloba*	EU101329	EU101374
Aplosporella papillata	CBS 121782	*Acacia erioloba*	EU101330	EU101375
Aplosporella prunicola^{T}	CBS 121167	*Prunus persica* var. *nucipersica*	KF766147	N/A
Aplosporella prunicola	STE-U 6326	*Prunus persica* var. *nucipersica*	EF564375	N/A
Aplosporella prunicola	STE-U 6327	*Prunus persica* var. *nucipersica*	EF564376	N/A
Aplosporella thailandica^{T}	MFLU 16-0615	N/A	KX423536	KX423537
Aplosporella yalgorensis	MUCC 511	*Eucalyptus gomphocephala*	EF591926	EF591977
Aplosporella yalgorensis^{T}	MUCC 512	*Mimetes cucullata* *Protea* sp	EF591927	EF591978
Saccharata proteae^{T}	CBS 115206	N/A	KC343004	KC343730

The new strains from the current study are in bold. Ex-type taxa are marked with a T.

Results

Phylogeny

The combined ITS and tef1-α dataset from 23 ingroup strains (sequences of two strains

from this study and sequences of 21 strains available in GenBank mostly from Mejía et al. 2011) clustered in 18 clades representing species of *Aplosporella* (Table 1). The alignment including gaps comprised 2864 characters of which 840 characters were constant, 467 variable characters were parsimony-uninformative, and 290 were parsimony informative. The heuristic search generated four equally parsimonious trees of 461 steps and CI = 0.911, RI = 0.839, RC = 0.764 as shown in Fig. 1. Strains CFCC 89961 and CFCC 89960, sequenced in this study, formed a well-supported clade (MP/BI = 100/1) representing a new phylogenetic species.

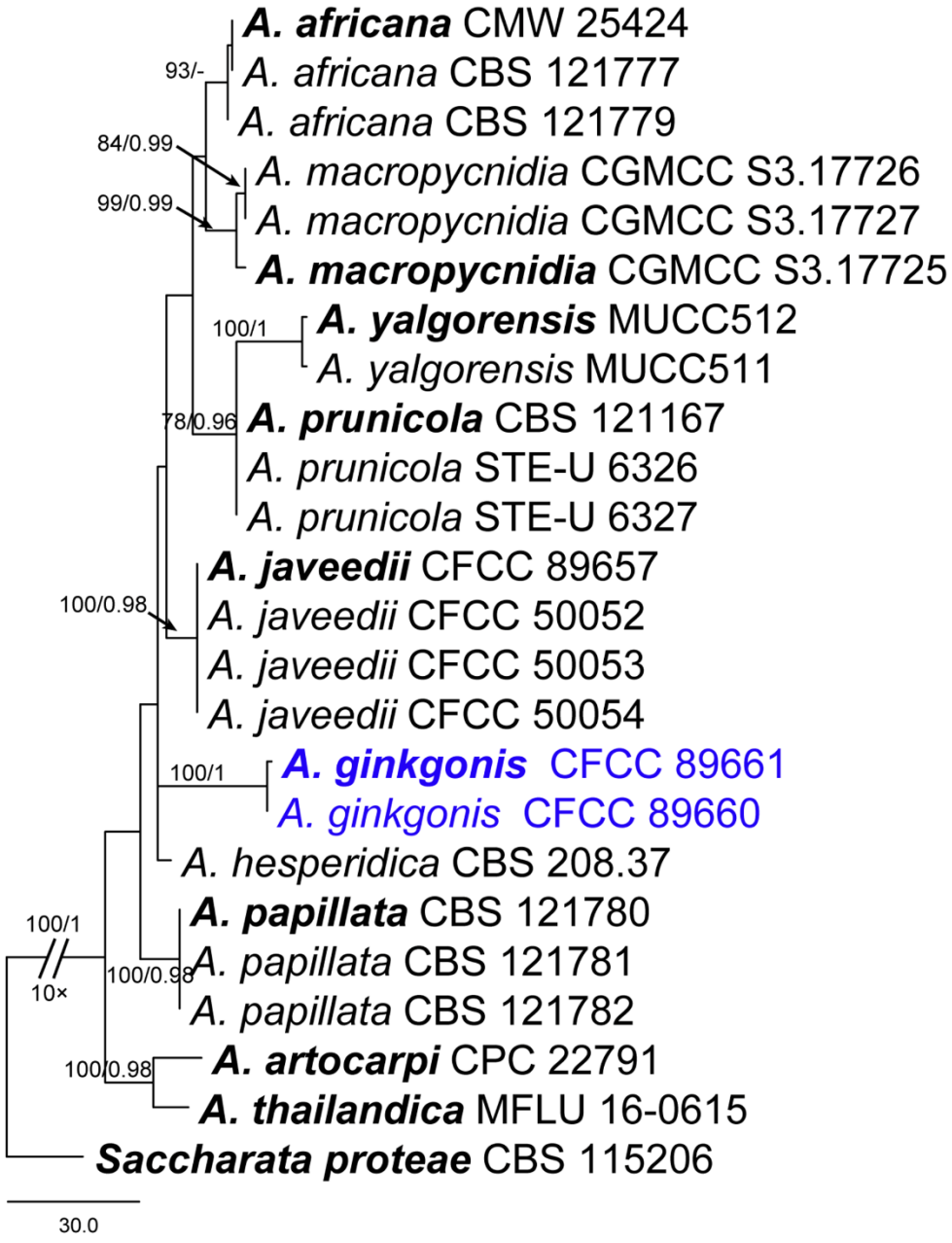

Figure 1 – Phylogram of *Aplosporella* based on analysis of combined ITS and tef1-α genes. MP bootstrap support values above 75 % and BI posterior probabilities support above 0.95 from BI are shown above the branches. Scale bar = 30 nucleotide substitutions. Ex-type strains are in bold. Strains in current study are in blue.

Taxonomy

Aplosporella ginkgonis C.M. Tian, Z. Du & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF552938; Facesoffungi number: FoF 02457

Etymology – Named after the host genus, *Ginkgo biloba*.

Holotype – BJFC-S939.

Host/Distribution – from branches of *Ginkgo biloba* in China.

Diagnoses – The asexual morph of *Aplosporella ginkgonis* is similar to *Aplosporella macropycnidia* Zh. P. Dou & Y. Zhang, which is from *Cerasus yedoensis* in China (Dou et al. 2017), but differs in its smaller pycnidia (59–63 × 77–81 μm vs. 45–180 × 38–138 μm).

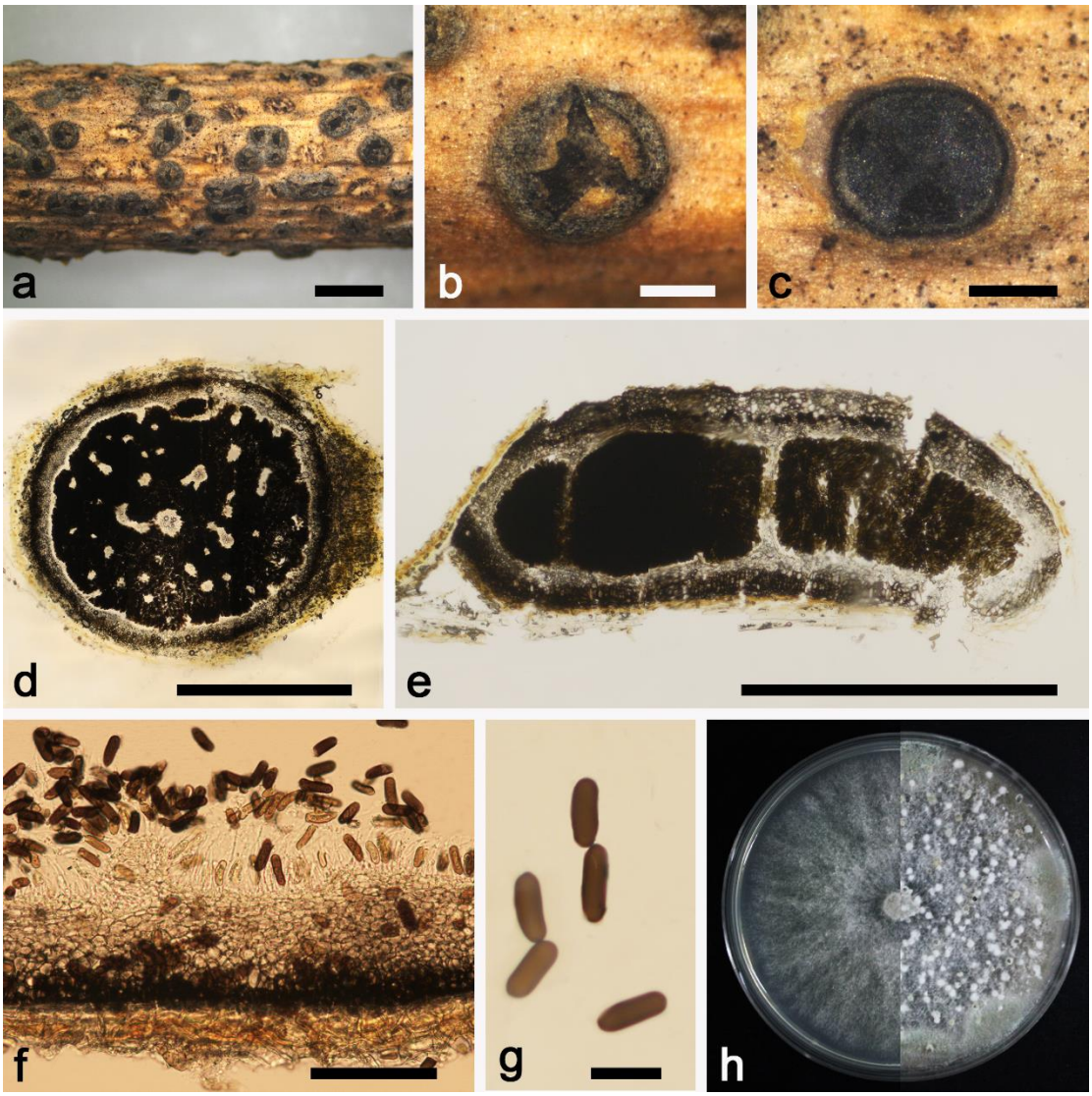

Figure 2 – Morphology of *Aplosporella ginkgonis*. a: Habit of conidiomata on twig. b: Multi-ostioles on the disc of conidiomata c, d: Transverse sections through conidiomata. e: Longitudinal sections through conidiomata. f: Conidiogenous cells and conidia. g: Conidia. h: Colonies on PDA at 3 days (left) and 30 days (right). Scale bars: a = 2 mm; b–e = 0.5 mm; f = 100 μm; g = 20 μm.

Sexual morph: Undetermined. ***Asexual morph***: *Conidiomatal stromata* immersed in bark, erumpent from surface of the bark, separate, discoid, dark-brown to black, multiloculate, up to 850 μm diam., wall consisting of 8–12 layers, outer layers thick composed of dark-brown *textura angularis*, becoming hyaline and thin-walled towards the inner region. *Disc* brown to black, circular to ovoid, (0.28–) 0.31–0.40(–0.44) mm (average = 0.36 mm, n = 20), with one to four ostioles per disc. *Ostioles* multiple, conspicuous, at the same level as the disc surface, covered below disc by lighter entostroma sometimes, (22.5–)23.9–30.7(–32.6) μm (average = 28 μm, n = 20) in diam. *Locules* multiple, irregularly arranged, subdivided frequently by invaginations with common walls, (0.59–)0.63–0.77(–0.81) mm (average = 0.71 mm, n = 20) in diam. *Conidiogenous*

cells formed from the cells lining the inner walls of the locules, hyaline, smooth, inconspicuous. *Conidia* aseptate, ellipsoid to oblong, smooth, ends rounded, initial hyaline, becoming brown when mature, (15.5–)16–20.5(–21) × (5.5–)6.0–7.5(–8) µm (average = 17.5 × 6.5 µm, n = 50).

Cultures – Cultures growth on PDA were initially white later producing a brown pigment on the reverse side after 7–10 days. Colonies were uniform with abundant white aerial mycelium.

Materials examined – CHINA. Gansu: Qingyang City, Qingyang Municipal Government, 35°42'23.21" N, 107°38'15.46" E, 1420 m asl., on stems of *Ginkgo biloba*, 14 Jul. 2013, collected by X.L. Fan (**holotype**, BJFC-S939, **ex-type** living culture, CFCC 89661); CHINA. Gansu, Qingyang City, Heshui County, 35°46'49.49"N, 107°59'31.48" E, 1277 m asl., on stems of *Morus alba*, collected by X.L. Fan, 13Jul. 2013, BJFC-S917, single conidium living culture CFCC 89660.

Notes – Morphologically, *Aplosporella ginkgonis* is characterized by its multilocular conidiomata with one to four ostioles, and aseptate, brown, ellipsoid to oblong conidia. Phylogenetically, two samples of *A. ginkgonis* formed a distinct lineage with high support (MP/BI = 100/1) in the combined ITS and tef1-α analysis. Strains CFCC 89660 and 89661 had been misidentified as *A. longipes* based on host association; however, these strains differ by their larger conidia (16–20.5 × 6–7.5 vs 12–20 × 5–6 µm) and represent a unique species of *Aplosporella* from *Ginkgo biloba* (Saccardo 1899). Both morphology and the sequence data confirmed that the two samples represent a new species in *Aplosporella* using the guidelines for new species in Jeewon & Hyde (2016).

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