Aplosporella prunicola, a novel species of anamorphic *Botryosphaeriaceae*

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Aplosporella prunicola is a newly described species associated with a dead branch of *Prunus persica* var. *nucipersica* from South Africa. Based on its phylogeny of the 28S rDNA (LSU) gene, the genus *Aplosporella* represents yet another anamorph lineage within the *Botryosphaeriaceae* (*Botryosphaeriales*). *Aplosporella* is characterized by having multilocular conidiomata opening by a single ostiole, verrucose, brown conidia, and the presence of prominent paraphyses. Details pertaining to the pathogenicity and host specificity of *Aplosporella* spp., remain to be elucidated.

Key words: *Botryosphaeria obtusa*, *Diplodia*, ITS, nomenclature, phylogeny, *Sphaeropsis*, taxonomy

Introduction

Aplosporella (lectotype: *A. chlorostroma* Speg.) has frequently been incorrectly cited as "*Haplosporella*" (Petrak and Sydow, 1927) and presently contains 325 epithets (April 2007: www.speciesfungorum.org). The name *Aplosporella* is, however, predated by *Podosporium* Bonord. (64 epithets), and it was suspected by Sutton (1980) that a formal proposal would be required to conserve *Aplosporella* Speg. over *Podosporium* Bonord. *Podosporium* Bonord (1851) is however, a homonym of *Podosporium* Lév. (1847) (= *Melampsora* Castagne) and *Podosporium* Schwein. (1832) (= dematiaceous hyphomycete, Ellis, 1971), thus it seems that *Aplosporella* is a suitable name for the genus.

Aplosporella is heterogeneous, containing several taxa that will need to be allocated elsewhere. Most species have been described based on their host occurrence, and although few data are presently available to refute this, it is unlikely that these species are highly host-specific. Most species presently

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known have been associated with thin, dead twigs, rarely occurring on leaves or thicker branches (Pande and Rao, 1995). Based on the type species, *Aplosporella* is circumscribed by having multilocular conidiomata opening by a communal ostiole, brown, aseptate, verrucose conidia, and filiform paraphyses (Sutton, 1980).

Several species of *Aplosporella* have been associated with teleomorphs such as *Bagnisiella* (*Dothideomycetes*), *Rhytidhysteron* (*Patellariaceae*), *Calospora* (= *Prosthecium*) (*Melanconidaceae*) and *Hypoxylina* (*Xylariaceae*), though none of these links have been proven in culture (Pande and Rao, 1995), and thus the correct affiliation of *Aplosporella* remains uncertain.

During the course of a study isolating fungi from fruit trees growing adjacent to vineyards in an attempt to locate alternate hosts for grapevine canker causing ascomycetes, an undescribed isolate of *Aplosporella* was obtained from *Prunus* wood. In the present study we name this organism, and also resolve the phylogenetic relationship of the genus *Aplosporella*.

Materials and methods

Isolates

Branches with symptomatic wood were sampled from nectarine (*Prunus persica* var. *nucipersica*) orchards in Modimolle (Nylstroom) (South Africa, Limpopo Province). Single conidial isolates from sporulating pycnidia were established on 2% tap water agar. Reference strains are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U) in Stellenbosch, South Africa, and the Centraalbureau voor Schimmelcultures (CBS) Utrecht, Netherlands.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on potato-dextrose agar (2% PDA, Biolab, Midrand, South Africa) plates following the protocol of Lee and Taylor (1990). The 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS-1 and ITS-2) and a partial sequence of the 28S rDNA gene (LSU) were amplified and sequenced using the primer pairs ITS-1F (Gardes and Bruns, 1993) + ITS-4 (White *et al*., 1990) and NL1 + NL4 (O'Donnell, 1993). The LSU sequences were added to the outgroup (*Magnaporte grisea* AF362554 and *Gaeumannomyces graminis* var. *avenae* AF362556) and sequences obtained from GenBank (http://www.ncbi.nlm.gov). The alignment was assembled and manually adjusted using Sequence

Alignment Editor v. 2.0a11 (Rambaut, 2002). Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2000). Neighbour-joining analyses were performed with the substitution models HKY85 as used in Crous *et al*. (2006) and a general time-reversible (GTR) substitution model with gamma (G) and proportion of invariable sites (I) as determined by Modeltest 3.5 (Posada and Crandall, 1998). Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Sequences derived in this study were lodged at GenBank, and the alignment in TreeBASE.

Morphology

The fungus was morphologically characterised on 2% tap water agar cultures with sterilised pine needles incubated at 25°C under near-ultraviolet light as described in Damm *et al*. (2007). Colony colours on PDA incubated at 25°C in the dark for 7 days were rated according to Rayner (1970).

Results

Phylogenetic analysis

For the LSU gene, 607 bases were determined for isolates STE-U6326 (EF564377) and STE-U6327 (EF564378). Additional sequences were obtained from GenBank and added to the LSU alignment that contained 93 taxa and 593 characters including the alignment gaps. Neighbour-joining analysis using the two substitution models yielded trees with similar topology and bootstrap values. The tree obtained with model HKY85 (Fig. 1) resulted in the same 12 clades as generated by Crous *et al*. (2006), with the exception that there is no bootstrap support for the *Diplodia*/*Lasiodiplodia* clade. With a bootstrap support of 85%, the two isolates of *Aplosporella prunicola* grouped with clades *Diplodia*/ *Lasiodiplodia*, *Botryosphaeria*, *Macrophomina*, *Neoscytalidium*, *Dothidotthia*, *Neofusicoccum*, *Pseudofusicoccum*, *"Botryosphaeria" quercuum*, *Saccarata* and *Guignardia*. They formed a separate clade (100% bootstrap) within this group.

For the ITS region, 566 bases were determined for isolates STE-U6326 (EF564375) and STE-U6327 (EF564376). BLASTn results of the ITS sequences had e-values of 2e-105 (94% identical) and 1e-103 (94% identical) with sequences of *Botryosphaeria corticis* (DQ299248) and *Botryosphaeria dothidea* (EF173913), respectively.

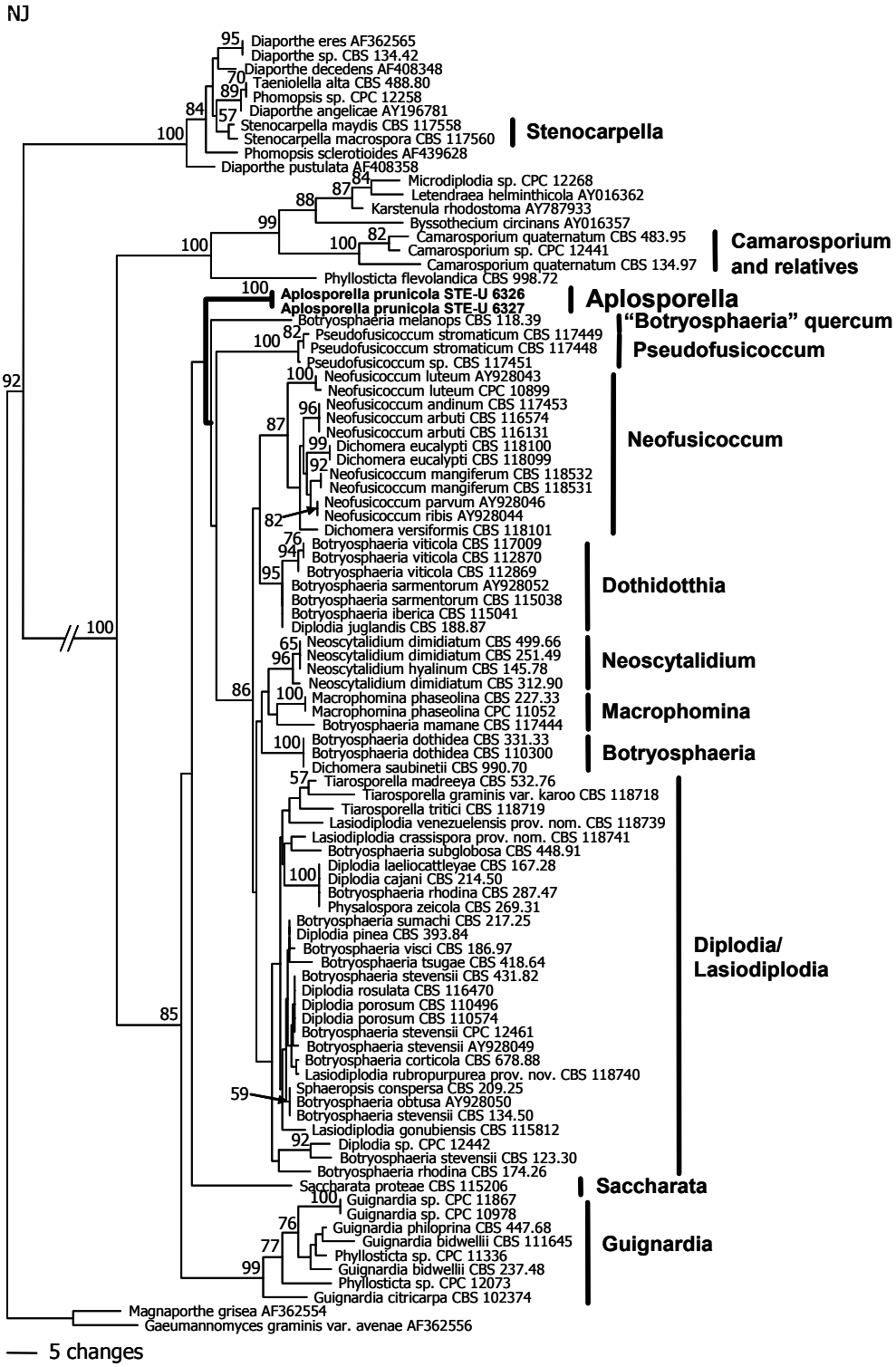
NJ
Diaporthe eres AF362565
Diaporthe sp. CBS 134.42
Diaporthe decedens AF408348
Taeniolella alta CBS 488.80
Phomopsis sp. CPC 12258
Diaporthe angelicae AY196781
Stenocarpella maydis CBS 117558
Stenocarpella macrospora CBS 117560
Stenocarpella
Phomopsis sclerotioides AF439628
Diaporthe pustulata AF408358
Microdiplodia sp. CPC 12268
Letendraea helminthicola AY016362
Karstenula rhodostoma AY787933
Byssothecium circinans AY016357
Camarosporium quaternatum CBS 483.95
Camarosporium sp. CPC 12441
Camarosporium quaternatum CBS 134.97
Camarosporium and relatives
Phyllosticta flevolandica CBS 998.72
Aplosporella prunicola STE-U 6326
Aplosporella prunicola STE-U 6327
Aplosporella
Botryosphaeria melanops CBS 118.39
"Botryosphaeria" quercum
Pseudofusicoccum stromaticum CBS 117449
Pseudofusicoccum stromaticum CBS 117448
Pseudofusicoccum sp. CBS 117451
Pseudofusicoccum
Neofusicoccum luteum AY928043
Neofusicoccum luteum CPC 10899
Neofusicoccum andinum CBS 117453
Neofusicoccum arbuti CBS 116574
Neofusicoccum arbuti CBS 116131
Dichomera eucalypti CBS 118100
Dichomera eucalypti CBS 118099
Neofusicoccum mangiferum CBS 118532
Neofusicoccum mangiferum CBS 118531
Neofusicoccum parvum AY928046
Neofusicoccum ribis AY928044
Dichomera versiformis CBS 118101
Neofusicoccum
Botryosphaeria viticola CBS 117009
Botryosphaeria viticola CBS 112870
Botryosphaeria viticola CBS 112869
Botryosphaeria sarmentorum AY928052
Botryosphaeria sarmentorum CBS 115038
Botryosphaeria iberica CBS 115041
Diplodia juglandis CBS 188.87
Dothidotthia
Neoscytalidium dimidiatum CBS 499.66
Neoscytalidium dimidiatum CBS 251.49
Neoscytalidium hyalinum CBS 145.78
Neoscytalidium dimidiatum CBS 312.90
Neoscytalidium
Macrophomina phaseolina CBS 227.33
Macrophomina phaseolina CPC 11052
Botryosphaeria mamane CBS 117444
Macrophomina
Botryosphaeria dothidea CBS 331.33
Botryosphaeria dothidea CBS 110300
Dichomera saubinetii CBS 990.70
Botryosphaeria
Tiarosporella madreeya CBS 532.76
Tiarosporella graminis var. karoo CBS 118718
Tiarosporella tritici CBS 118719
Lasiodiplodia venezuelensis prov. nom. CBS 118739
Lasiodiplodia crassispora prov. nom. CBS 118741
Botryosphaeria subglobosa CBS 448.91
Diplodia laeliocattleyae CBS 167.28
Diplodia cajani CBS 214.50
Botryosphaeria rhodina CBS 287.47
Physalospora zeicola CBS 269.31
Botryosphaeria sumachi CBS 217.25
Diplodia pinea CBS 393.84
Botryosphaeria visci CBS 186.97
Botryosphaeria tsugae CBS 418.64
Botryosphaeria stevensii CBS 431.82
Diplodia rosulata CBS 116470
Diplodia porosum CBS 110496
Diplodia porosum CBS 110574
Botryosphaeria stevensii CPC 12461
Botryosphaeria stevensii AY928049
Botryosphaeria corticola CBS 678.88
Lasiodiplodia rubropurpurea prov. nov. CBS 118740
Sphaeropsis conspersa CBS 209.25
Botryosphaeria obtusa AY928050
Botryosphaeria stevensii CBS 134.50
Lasiodiplodia gonubiensis CBS 115812
Diplodia sp. CPC 12442
Botryosphaeria stevensii CBS 123.30
Botryosphaeria rhodina CBS 174.26
Diplodia/ Lasiodiplodia
Saccharata proteae CBS 115206
Saccharata
Guignardia sp. CPC 11867
Guignardia sp. CPC 10978
Guignardia philoprina CBS 447.68
Guignardia bidwellii CBS 111645
Phyllosticta sp. CPC 11336
Guignardia bidwellii CBS 237.48
Phyllosticta sp. CPC 12073
Guignardia citricarpa CBS 102374
Guignardia
Magnaporthe grisea AF362554
Gaeumannomyces graminis var. avenae AF362556
5 changes

Fig. 1. Distance tree using the HKY85 substitution model on the LSU alignment. Bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to *Magnaporthe grisea* (AF362554) and *Gaeumannomyces graminis* var. *avenae* (AF362556).

Morphology

Aplosporella prunicola Damm & Crous, **sp. nov.** (Fig. 2)
MycoBank: 504373

Aplosporellae pruni similis, sed conidiis minoribus, (17-)19- 22(25) × (9-)10-12(-18) µm, in medio 20 × 11 µm.

Conidiomata pycnidial, sporulating readily on pine needles on WA in 2–4 weeks, solitary, dark brown, 400-800 × 200-350 µm, immersed to semi-immersed, erumpent, multilocular, locules divided by pale brown *textura prismatica*, ostiole central, 60-80 µm diam; wall 6-10 cell-layers thick (75-150 µm), outer layers composed of dark-brown *textura angularis*, becoming hyaline towards the inner region. Exposed parts setose; setae hyaline to pale brown, smooth or verrucose, 3-5 × 10-120 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, hyaline, cylindrical to doliiform, smooth-walled, proliferating percurrently with 1-3 annellations near the apex, 6-11 × 2.5-3 µm. *Paraphyses* hyaline, smooth-walled, septate, branched below, 35-95 µm long, 4-8 µm wide at the base, 1-3 µm wide in the upper part. *Conidia* aseptate, initially hyaline, smooth-walled, broadly ellipsoidal to subcylindrical, with rounded ends, becoming dark brown (black in mass), prominently spinulose before discharge from pycnidia, spines up to 1.2 µm long (wall with spines up to 1.5 µm thick), (17-)19-22(25) × (9-)10-12(-18) µm, mean ± SD = 20.2 ± 1.3 × 11 ± 0.8 µm, L/W ratio = 1.8.

Cultural characteristics: Colonies on PDA after 2 weeks in the dark: olivaceous to grey-olivaceous in the centre, olivaceous-buff to greenish-olivaceous towards the margin, similar in reverse; aerial mycelium appressed, floccose, white to smoke-grey. Colonies flat with undulate edge, 38 mm diam after 2 days, reaching the edge of the Petri dish within 4 days.

Teleomorph: Unknown *Botryosphaeriaceae*.

Habitat: In *Prunus* wood.

Known distribution: South Africa.

Hosts: *Prunus persica* var. *nucipersica*.

Distribution: Modimolle (Nylstroom) (South Africa, Limpopo Province).

Specimens examined: SOUTH AFRICA, Limpopo Province, Modimolle, from bark of small dead tree of *P. persica* var. *nucipersica*, 30 August 2004, U. Damm, CBS-H 19848 (**holotype**), culture ex-type CBS 121167 = STE-U 6326; Limpopo Province, Modimolle, from the same specimen of *P. persica* var. *nucipersica*, STE-U 6327.

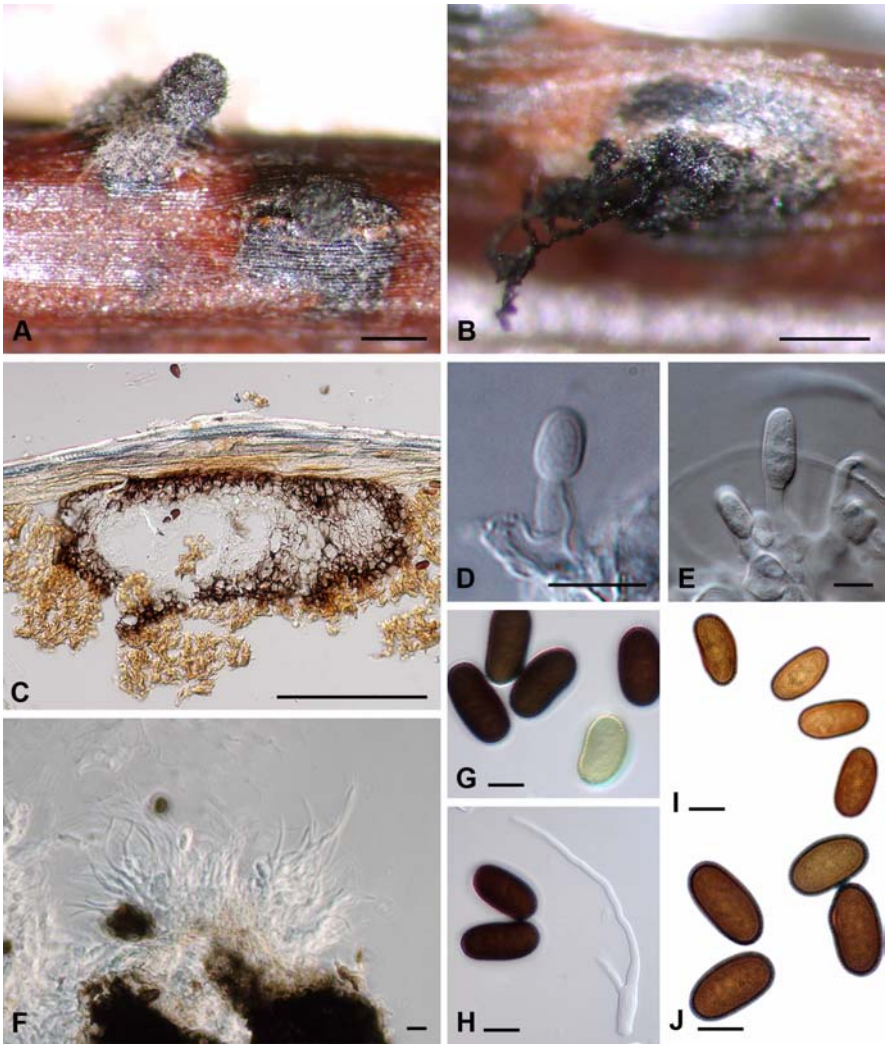

Fig. 2. *Aplosporella prunicola*. **A, B.** Oozing spore masses from submerged pycnidia. **C.** Transverse section though multilocular conidioma. **D, E**. Conidiogenous cells. **F.** Paraphyses. **H.** Conidia and branched paraphyses. G, I, J. Conidia. Bars: A–C = 250 µm. F = 20, D, E, G–J = 10 µm.

Discussion

Botryosphaeria Ces. & De Not. is a species-rich genus with a wide host range, though a few taxa are known that appear to be rather host-specific (Phillips *et al*., 2006; Summerell *et al*., 2006; Slippers *et al*., 2007). In a recent phylogenetic study, Crous *et al*. (2006) revised the complex, recognizing up to nine different genera, and restricting *Botryosphaeria* to those taxa with

Fusicoccum Corda anamorphs allied to *B. dothidea* (Moug. : Fr.) Ces. & De Not. Several anamorph genera that were fusicoccum-like were treated, namely *Tiarosporella* Höhn., *Neofusicoccum* Crous, Slippers & A.J.L. Phillips and *Pseudofusicoccum* Mohali, Slippers & M.J. Wingf., with a few more awaiting formal description. Dark spored anamorphs were primarily accommodated in *Diplodia* Fr., *Lasiodiplodia* Ellis & Everh., *Macrophomina* Petr. and *Dothiorella* Sacc. (Alves *et al*., 2006; Phillips *et al*., 2006; 2007). In a step away from dual nomenclature, only a single generic name was proposed per clade, which in some cases was a teleomorph genus, but in others, like *Lasiodiplodia* and *Dothiorella* for instance, preference was given to the anamorph name. In recent treatments of *Aplosporella*, Sutton (1980) and Agarwal *et al*. (1992) did not elude to its affinity, and those purported teleomorphs listed by Pande and Rao (1995) were never confirmed in culture, and are scattered throughout the Ascomycota. The phylogenetic placement of *A. prunicola* within the *Botryosphaeriaceae* (*Botryosphaeriales*) (Crous *et al*., 2006; Schoch *et al*., 2006) adds yet another anamorph genus to this important complex of canker pathogens. However, no *Botryosphaeriaceae* teleomorphs have thus far been confirmed for any species of *Aplosporella*, and details pertaining to their potential pathogenicity also need further elucidation.

Aplosporella species have mostly been described as new, chiefly based on their host occurrence. The isolate of *Aplosporella prunicola* was isolated from symptomatic wood of *Prunus* and the key provided by Pande and Rao (1995) keyed out to *A. malorum* Sacc. (on apple in temperate regions) and *A. marathwadensis* Tilak & R. Rao (other hosts in tropical regions). Conidia of *A. malorum* (16.5-20 × 6.6-10 μm) are much narrower, while those of *A. marathwadensis* (8-12 × 3-5 μm) are again much smaller than those of *A. prunicola*. Other species reported on *Prunus* are *A. phyllanthina* Syd. (12-18 × 9-12.5), and *A. pruni* McAlpine (22-24 × 13-15 μm), which show some overlap in conidial dimensions, but are respectively smaller and larger than the averages observed for *A. prunicola*. As far as could be established without revising all taxa in the genus, *A. prunicola* appears to be a novel species occurring on *Prunus* wood in South Africa. Although it was isolated from a dead branch, we have not determined the pathogenicity of *A. prunicola*. Furthermore, as it was encountered only once during our surveys, its potential host range also remains unclear. Knowing that *Aplosporella* belongs to the *Botryosphaeriaceae*, we hope that future studies would reveal more species on other hosts, and provide us with a better understanding of the host specificity and potential pathogenicity of *Aplosporella* species.

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