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 Gaemammomyces 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, Dothinotthia, 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.
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.*


**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
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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 (Botryosphaerales) (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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References


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