
Two new species of Diatrypaceae from coastal wattle in Coorong National Park, South Australia

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In the present study, two species of Diatrypaceae were isolated from the wood of *Acacia longifolia* subsp. *sophorae* shrubs in the Coorong National Park, South Australia. Based on habitat, host, morphological observations and literature review, the isolates are described as the new species *Diatrype brunneospora* and *Eutypella australiensis*. These new taxa are fully described and illustrated and sequences of the internal transcribed spacer region of the nuclear ribosomal DNA are also provided.

Key words – Australia – Diatrypaceae – New species – Taxonomy

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Introduction

Species of Diatrypaceae (Xylariales) are commonly found on stems of various woody plants around the world. They are generally considered to be saprotrophs, although some species seem to be especially well established in wood of recently dead host plants (Tiffany & Gilman 1965). A few species in this family constitute serious plant pathogens, among them *Eutypa lata* (Pers.) Tul. & C. Tul. (syn.: *E. armeniaca* Hansf. & Carter) has been particularly well investigated as the causal agent of Eutypa dieback of several woody crops worldwide (Carter 1991). *Cryptosphaeria lignyota* (Fr.) Auersw. (syn.: *C. populina* (Pers.) Sacc.) and *Eutypella parasitica* R.W. Davidson & R.C. Lorenz in this family represent severe pathogens of forest trees in the United States and Europe (Davidson and Lorenz 1938, Hinds 1981, Jurc et al. 2006).

The taxonomy of octosporous Diatrypaceae has been revised and descriptions of approximately 120 morphological species have

been made (Rappaz 1987). Among them, eight genera have been recognized (Rappaz 1987); these include *Cryptosphaeria* Ces. & De Not. (four species), *Diatrype* Fr. (56 species), *Dothi-
deovalsa* Speg. (three species), *Echinomyces* F. Rappaz (two species), *Eutypa* Tul. & C. Tul. (26 species), *Eutypella* (Nitschke) Sacc. (76 species), *Leptoperidia* F. Rappaz (four species) and *Rostronitschkia* Fitzp. (one species).

In North America, the works of Glawe and Rogers (1984), and Tiffany and Gilman (1965), constitute important additional taxonomic studies of Diatrypaceae. Recently, Vasilyeva and Stephenson (2004, 2005, 2006, 2009) described various species of Diatrypaceae from the Great Smoky Mountains National Park in the eastern United States, as well as Arkansas and Texas. In California, 11 diatrypaceous species were newly reported and described from *Vitis vinifera* and various other woody host plants (Trouillas & Gubler 2004, Trouillas et al. 2010). The diversity of Diatrypaceae from Argentina was also recently investigated

and new species as well as new records were proposed (Carmaran et al. 2009).

The current generic delineation and classification in the Diatrypaceae as suggested by Rappaz (1987) is based primarily on characters of the teleomorphic states, including stroma morphology and organization of perithecia. However, much overlap of taxonomic characters exists between the current diatrypaceous genera. Vasilyeva and Stephenson (2004) acknowledged that the concept of *Diatrype* as delimited by Rappaz (1987) is somewhat problematic, as there is, in some instances, no clear separation between this genus and either *Eutypa* or *Eutypella*. Moreover, phylogenetic classifications of Diatrypaceae were attempted using molecular data as well as morphological characters but evolutionary relationships in the family remained uncertain (Acero et al. 2004, Carmaran et al. 2006, Trouillas et al. 2010).

The diatrypaceous mycota of Australia is remained largely unexplored. A few species have been studied, mainly because of their occurrence on *Vitis vinifera* grapevines (Carter 1991, Mostert et al. 2004, Pitt et al. 2009). The occurrence of Diatrypaceae on native Australian host plants has been given little consideration. In his account of Australian fungi, Cooke (1892) reported nine putative species of Diatrypaceae. An additional three species were described from intertidal host plants in north Queensland: *Cryptovalsa halosarceicola* K.D. Hyde on *Halosarcia halocnemoides* in a mangrove at Cairns Airport (Hyde 1993a), *Eutypa bathurstensis* K.D. Hyde & Rappaz (Hyde 1993b) and *Eutypella naqsii* K.D. Hyde (Hyde 1995) on *Avicennia* sp. at Bathurst Heads. In a study of fungi associated with *Acacia* and *Eucalyptus* plants in Melville Island in the Northern Territory of Australia, Yuan (1996) reported *Cryptovalsa protracta* (Pers.) De Not., *Diatrype stigma* (Hoffm.) Fr. and *Eutypella scoparia* (Schwein.) Ellis & Everh. To our knowledge, the above references constitute the only studies that illustrate the diatrypaceous biota in Australia.

During the course of surveys for Diatrypaceae associated with *Vitis vinifera* in South Australia, two original diatrypaceous species were collected on native *Acacia longifolia* subsp. *sophorae* (coastal wattle) Fabaceae) from Coorong National Park. The

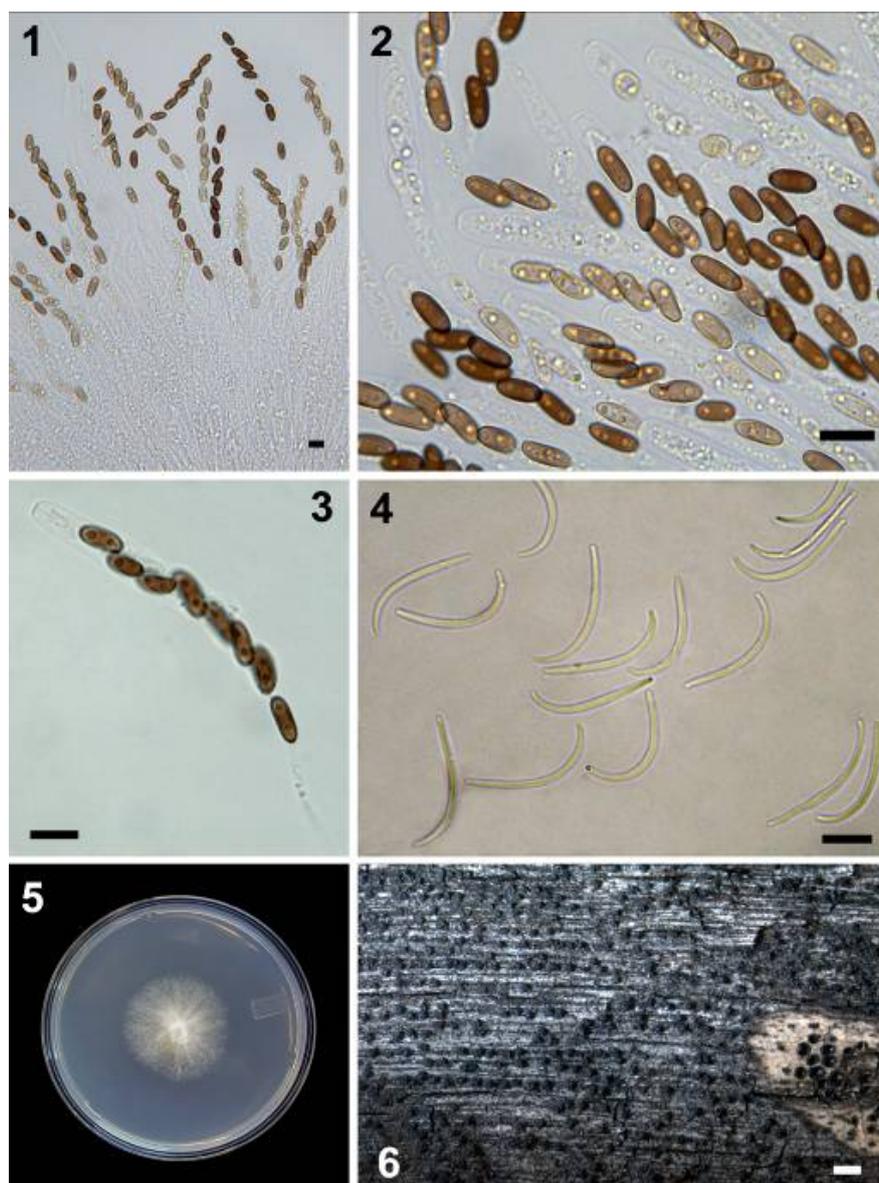
Coorong is a wetland ecosystem and estuary, particularly valuable for its unique ecology and cultural significance. *Acacia longifolia* subsp. *sophorae* is a common shrub indigenous to south eastern Australia and restricted mainly to coastal dunes. The taxa isolated for this study differed from known species in various aspects of ascospores and ascomata morphology, in habitat and host, and deserve description as new species.

Methods

Pieces of dead stems of *Acacia longifolia* subsp. *sophorae* with black and effuse stromata typical of Diatrypaceae were collected in December 2008 from the Coorong National Park in South Australia. Microscopic analyses and photography were conducted using standard light microscopy with a Leica DMLB microscope mounted with a Leica DFC480 digital camera. Stroma and perithecial characteristics were examined using a Leica MZ95 stereo microscope. Examinations and measurements of microscopic characters were carried out in water. Melzer's reagent also was used to assess amyloid reaction of the apical ascus ring. Thirty ascospores, ten asci (spore bearing portion only) and ten perithecia of each species isolate were measured. Pure fungal cultures were obtained from hyphal tips and grown on potato dextrose agar (PDA) (Difco Laboratories, Detroit) dishes incubated under 24-hours fluorescent lighting. Thirty conidia per isolate were measured in water as conidial masses became visible from colonies. Sequences of the nuclear internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) were obtained following standard DNA extraction procedure and amplification by polymerase chain reaction (PCR) using primers ITS1 and ITS4 (White et al. 1990). Sequences have been deposited into GenBank. Holotypes and ex-type cultures were deposited at the Australian Scientific Collections Unit, NSW Industry & Investment, Orange, NSW, Australia (DAR).

Taxonomy

Diatrype brunneospora Trouillas, Sosnowski & Gubler, **sp. nov.** Figs 1–6
Mycobank 518611

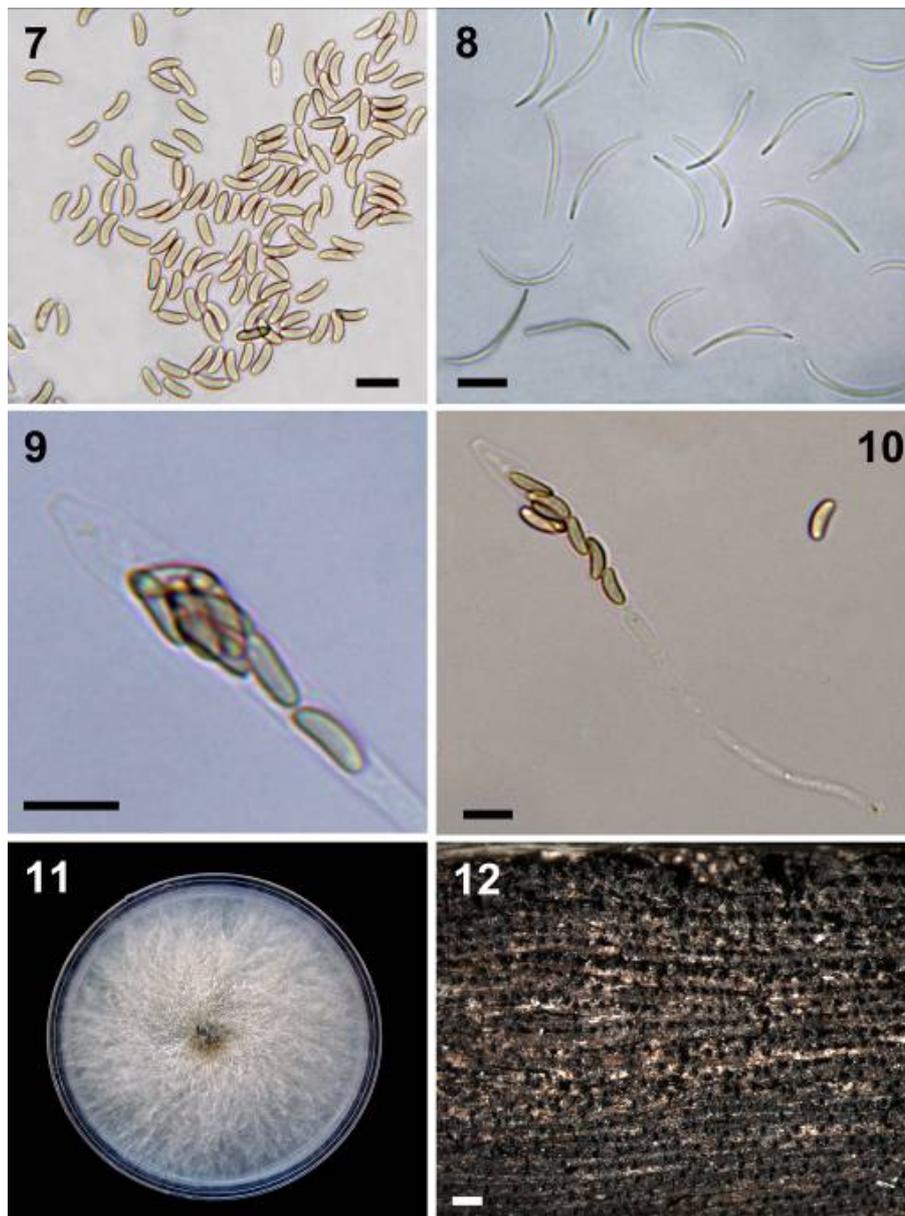


Figs – 1–6 – Morphology of *Diatrype brunneospora*. 1. Asci and paraphyses. 2. Mature (brown) and immature (light brown) ascospores showing typical oil drops. 3. Cylindrical to clavate ascus, with flat apex. 4. Filiform conidia. 5. Colony after 6 days on PDA dishes (85 mm diam) incubated under 24-hours fluorescent lighting. 6. Perithecial cavities and emerging perithecial ostioles in the wood of *Acacia longifolia* subsp. *sophorae*. Bars = 10 μ m in 1–4; 1 mm in 6.

Etymology – referring to the brown ascospores.

Stromata lata effusa, in ligno decorticato. Perithecia immersa, monosticha, globosa vel ovoidea, 0.4–0.5 mm diam, ostiolis quadrisulcatis, 0.3–0.4 mm diam. Asci octospori, clavulato-cylindraceuti, parte sporifera 65–85 \times 6–7.5 μ m, annulis apicalibus haud amyloideis ornati. Ascopora oblongae, brunneae, oleosae, (6.5–)8–10(–12.5) \times 3.5–4.5 μ m. Conidia filiforma, subhyalina, (20–)22–32(–34) \times 1–1.5 μ m. Hab. in ramis emortuis *Acacia longifolia* subsp. *sophorae*, in South Australia.

Stromata effuse, embedded in decorticated wood and incorporating host tissues, rather eutypoid, blackening the host surface. Perithecia in one layer, globose to ovoid, irregularly scattered, 0.4–0.5 mm diam, ostioles commonly 4-sulcate, occasionally 5-sulcate, generally emerging separately, 0.3–0.4 mm diam. Asci cylindrical to clavate with flat apex and easily breaking stipes, without amyloid reaction in Melzer's reagent, 8-spored, p. sp. 65–85 \times 6–7.5 μ m. Ascospores oblong, dark brown at maturity, usually with two oil droplets, (6.5–)8–10(–12.5) \times 3.5–4.5 μ m.



Figs – 7–12 – Morphology of *Eutypella australiensis*. 7. Allantoid subolivaceous ascospores. 8. Filiform conidia. 9. Knobbed apex of an ascus. 10. Clavate and long-stipitate ascus. 11. Colony after 6 days on PDA dishes (85 mm diam) incubated under 24-hours fluorescent lighting. 12. Emerging perithecial ostioles in the wood of *Acacia longifolia* subsp. *sophorae*. Bars = 10 μ m in 7–10; 1 mm in 12.

Cultural characteristic – Colonies white on PDA, growing slowly and covering half (with apparently no further increase in colony size) of an 85 mm Petri dish after 20 days. Conidia filiform, subhyaline, $17\text{--}28 \times 1\text{--}1.5 \mu\text{m}$.

Known distribution – South Australia.

Material examined – AUSTRALIA, SOUTH AUSTRALIA, Coorong National Park, along Princes Highway, on dead wood of *Acacia longifolia* subsp. *sophorae* (Labill.) Court, December 2008, **holotype**: F.P.

Trouillas CNP01, herb. and ex-type culture = DAR 80711, GenBank accession no for ITS rDNA sequence: HM581946.

Notes – Because of the taxonomic confusion around Diatrypaceae and the difficulty in segregating the various genera, the assignment of this isolate to the genus *Diatrype* may require reconsideration in the future. This fungus has morphological similarity with members of the genus *Eutypa*. However, based on preliminary phylogenies, this fungus appears best placed in *Diatrype*.

Eutypella australiensis Trouillas, Sosnowski & Gubler, **sp. nov.** Figs 7–12

MycoBank 518612

Etymology – referring to the Australian origin.

Stromata lata effusa, in ligno decorticato. Perithecia immersa, nonnunquam exposita, monosticha vel polysticha, globosa vel ovoidea, ostiolis trisulcatis. Asci octospori, claviti, longe stipitati, annulis apicalibus haud amyloideis ornate, parte sporifera 40–50 × 7–8.5 µm. Ascopora allantoideae, lutescentiae vel olivascentiae. Conidia filiforma, subhyalina, (16–)18–22(–25) × 1–1.5 µm. Hab. in ramis emortuis *Acacia longifolia* subsp. *sophorae*, in South Australia.

Stromata poorly developed, wide spreading, on decorticated wood surface. Perithecia in contact, in one or two layers, globose to ovoid, raising the outer wood layer and eventually becoming exposed, 0.25–0.4 mm diam, with short perithecial necks, ostioles commonly 3-sulcate, emerging mostly separately or in groups of two, 0.1–0.2 mm in diameter. Asci clavate, with apex more or less knobbed, long-stipitate, without amyloid reaction in Melzer's reagent, 8-spored, p. sb. 40–50 × 7–8.5 µm. Ascospores allantoid to suballantoid, yellowish to subolivaceous, 8–10 × 3 µm.

Cultural characteristic – Colonies white on PDA, covering 85 mm Petri dish after 5 days, mycelium later turning grey, beginning from the center of the colony. Conidia filiform, subhyaline, (16–)18–22(–25) × 1–1.5 µm.

Known distribution – South Australia.

Material examined – AUSTRALIA, SOUTH AUSTRALIA, Coorong National Park, along Princes Hwy, on dead wood of *Acacia longifolia* subsp. *sophorae* (Labill.) Court, December 2008, **holotype**: F.P. Trouillas CNP03, herb. and ex-type culture = DAR 80712, GenBank accession no for ITS rDNA sequence: HM581945.

Notes – Because of the taxonomic confusion around Diatrypaceae and the difficulty in separating the various genera, the assignment of this isolate to the genus *Eutypella* may require reconsideration in the future. Based on preliminary phylogenies, this fungus appears best placed in *Eutypella*.

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