

Taxonomy and DNA phylogeny of Diatrypaceae associated with *Vitis vinifera* and other woody plants in Australia

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Abstract The Diatrypaceae occur worldwide and comprise a number of pathogens of woody crops, forest and ornamental tree species. Despite the taxonomic difficulties within this family, interest in the Diatrypaceae has increased recently, mainly due to the recent detection of these fungi in the premium grape growing regions of California. In the present study, we investigated the diversity and host range of diatrypaceous fungi from prominent wine grape growing regions in South Australia, New South Wales and Western Australia. Approximately 100 isolates were collected from grapevine and other woody plants and compared with reference collections from the United States and Europe. Phylogenetic analyses of the complete sequence of the internal transcribed spacer (ITS) region of the ribosomal DNA and partial sequence of the β -tubulin gene, combined with morphological analyses separated 12 species. These included the previously described species *Cryptovalsa ampelina*, *C. rabenhorstii*, *Diatrype*

brunneospora, *Eutypa lata*, *E. leptoplaca*, *Eutypella australiensis*, *E. citricola*, a *Cryptosphaeria* sp. and a *Diatrype* sp., whereas *Diatrypella vulgaris*, *Eutypella cryptovalsoidea* and *E. microtheca* are described as new. Seven species were isolated from grapevine but the prevalence of Diatrypaceae in grapevine cankers varied among the regions surveyed. In many instances in WA and NSW, these newly reported fungi were more widespread and abundant than *E. lata*. This study provides new information to assist with diagnosis of the causal agents of dieback and canker diseases in Australia and development of management strategies. Further studies to characterize the pathogenicity of diatrypaceous species to grapevines and to elucidate the biology of these fungi are underway.

Keywords *Cryptovalsa* · Diatrypaceae · Diatrypella · *Eutypa* dieback · *Eutypella*

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Introduction

Species of Diatrypaceae (Xylariales) are widespread inhabitants of dead wood and bark of a broad variety of plants around the world. Principal morphological characteristics of Diatrypaceae consist of perithecial ascomata embedded usually in a black-colored stroma, long stalked asci and allantoid ascospores (Glawe and Rogers 1984; Rappaz 1987). The current edition of Dictionary of the Fungi reports 13 genera and more than 220 morphological species (Kirk et al. 2008), with the most common genera comprising *Cryptosphaeria* Ces. & De Not., *Cryptovalsa* (Ces. & De Not.), *Diatrype* Fr., *Diatrypella* (Ces. & De Not.) De Not., *Eutypa* Tul. & C. Tul., and *Eutypella* (Nitschke) Sacc.

While several species, such as *Cryptovalsa ampelina* (Nitschke) Fuckel, *Eutypa lata* (Pers.: Fr.) Tul. & C. Tul. and *E. leptoplaca* (Mont.) Rappaz, are cosmopolitan (Carter 1991; Trouillas and Gubler 2004; Trouillas et al. 2010a, b), others, most notably *Diatrype disciformis* (Hoffm. : Fr.) Fr. are thought to be extremely rare outside Europe (Rappaz 1987). Furthermore, some species appear to be associated with a specific host, for instance *Eutypa maura* (Fr. : Fr.) Fuckel on *Acer pseudoplatanus* (Rappaz 1987), while others, specifically *E. lata*, *E. leptoplaca* and *C. ampelina* demonstrate wider host ranges (Carter et al. 1983; Rappaz 1987; Trouillas and Gubler 2004; Trouillas and Gubler 2010; Trouillas et al. 2010a, b). Regardless, species within the Diatrypaceae have, for the most part, been considered saprotrophic, although some species appear to be especially well established in the wood of recently dead host plants (Tiffany and Gilman 1965). Nevertheless, a few species in this family are known as severe plant pathogens of woody crops, landscape and forest trees in the United States (US) and Europe (Carter 1957; Carter 1991; Davidson and Lorenz 1938; Hinds and Laurent 1978; Hinds 1981; Moller and Kasimatis 1978; Munkvold and Marois 1994; Sinclair and Lyon 2005; Jurc et al. 2006). Among those of economical importance, *E. lata* has been studied extensively both in Australia and around the world as the causal agent of Eutypa dieback of grapevine (*Vitis vinifera* L.) and apricot (*Prunus armeniaca* L.) (Carter 1957; Carter 1991).

The biodegradation potential of diatrypaceous strains was recently investigated (Pildain et al. 2005). This study has shown that some members of the Diatrypaceae family produce cellulase and lignin-degrading enzymes, extracellular enzymes that catalyse the hydrolysis of cellulose and breakdown of lignin in the cell walls of plants, thus affording some species the physiological capacity to produce wood decay (Pildain et al. 2005). Recent studies in the US reported several species as putative pathogens of grapevine (Rolshausen et al. 2004; Catal et al. 2007; Trouillas and Gubler 2004; Trouillas and Gubler 2010;

Trouillas et al. 2010a, b; Úrbez-Torres et al. 2009). *Eutypella vitis* (Schwein.:Fr.) Ellis and Everh. [syn.: *E. aequilinearis* (Schwein.:Fr.) Starb.] and *Diatrypella* sp. were shown to be somewhat pathogenic to grapevine in Texas (Úrbez-Torres et al. 2009). In California, *E. leptoplaca*, *Diatrype stigma* (Hoffm. : Fr.) Fr., *D. whitmanensis* J.D. Rogers & Glawe, *Cryptosphaeria pullmanensis* Glawe and *C. ampelina* were shown to infect grapevine wood, causing decay of vascular tissues (Trouillas and Gubler 2004; Trouillas and Gubler 2010). Californian studies also suggested a possible correlation between grapevine infection by diatrypaceous species and the immediacy of fungal fruiting bodies (perithecia) on natural and ornamental host plants in the surrounding ecosystems, often in close proximity to vineyards (Trouillas and Gubler 2010; Trouillas et al. 2010a, b).

Berlese (1900) introduced the earliest large-scale taxonomic study of Diatrypaceae, providing excellent illustrations for many species. Rappaz (1987) revised the family examining thoroughly original descriptions and types around the world. To date, his work provides the most comprehensive treatment on the taxonomy of octosporous Diatrypaceae. In North America, Ellis and Everharts (1892) proposed descriptions for numerous Diatrypaceae, including polysporous genera. Later, Tiffany and Gilman (1965), and Glawe and Rogers (1984), described Diatrypaceae from Iowa and from the Pacific Northwest, respectively. Lately, Vasilyeva and Stephenson (2004, 2005, 2006, 2009) described several species from the Great Smoky Mountains National Park in the eastern US, Arkansas and Texas. Additional studies have investigated the diversity of Diatrypaceae in Argentina, describing new species and new records (Romero and Carmarán 2003; Carmarán et al. 2009).

The current generic delineation and classification of Diatrypaceae as proposed by Rappaz (1987) is based primarily on characters of the teleomorphic states, including stroma morphology and organization of perithecia. However, much overlap of these taxonomic features exists among the current diatrypaceous genera. For example, the concept of *Diatrype* as delimited by Rappaz (1987) has, in some instances, no clear separation from either *Eutypa* or *Eutypella* (Vasilyeva and Stephenson 2004). Overall, the taxonomy of the Diatrypaceae is outdated making the identification of these fungi particularly difficult. Published diagnoses for these species are often vague and incomplete, while most original descriptions as well as types are largely inaccessible or lost. The current classification of diatrypaceous genera remains provisional and there is an urgent need to revise the classification of the family and test the significance of generic concepts using molecular phylogeny. Preliminary attempts at phylogenetic classification using molecular data as well as morphological characters remained

Table 1 Isolates collected for this study and used either in the morphological or phylogenetic studies

Collection number	Species	Host	Origin	Collector/Isolator	CBS accession no.	DAR accession no.	ITS rDNA GenBank accession no.	β -tubulin GenBank accession no.
NSW05PO ^a	<i>Cryptosphaeria</i> sp.	<i>Populus balsamifera</i>	Khancoban, New South Wales	F.P. Trouillas			HQ692618	HQ692508
B10-16A ^a	<i>Cryptovalsa ampelina</i>	<i>Vitis vinifera</i>	South Australia	M.R. Sosnowski/A. Loschiavo			HQ692547	HQ692472
ADSC200	<i>C. ampelina</i>	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, South Australia	F.P. Trouillas			HQ692546	HQ692458
AD100	<i>C. ampelina</i>	<i>Vitis vinifera</i>	South Australia	F.P. Trouillas			HQ692551	HQ692468
C14A ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	South Australia	M.R. Sosnowski/A. Loschiavo			HQ692550	HQ692473
C17A ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	South Australia	M.R. Sosnowski/A. Loschiavo			HQ692549	HQ692474
B2-15A ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	South Australia	M.R. Sosnowski/A. Loschiavo			HQ692548	HQ692471
RGA05 ^a	<i>C. ampelina</i>	<i>Fraxinus angustifolia</i>	Adelaide hills, South Australia	F.P. Trouillas			HQ692552	HQ692475
ABA100	<i>C. ampelina</i>	<i>Fraxinus angustifolia</i>	Barossa Valley, South Australia	F.P. Trouillas			HQ692540	HQ692470
AH01	<i>C. ampelina</i>	<i>Acer macrophyllum</i>	Adelaide Hills, South Australia	F.P. Trouillas			HQ692553	HQ692469
SAPN03	<i>C. ampelina</i>	<i>Populus nigra</i> 'italica'	McLaren Flat, South Australia	F.P. Trouillas			HQ692555	HQ692461
TUUP01	<i>C. ampelina</i>	<i>Ulmus procera</i>	Tumbarumba, New South Wales	F.P. Trouillas			HQ692543	HQ692463
HVVIT04	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	F.P. Trouillas			HQ692558	HQ692459
CSU01	<i>C. ampelina</i>	<i>Pistacia vera</i>	Wagga Wagga, New South Wales	F.P. Trouillas			HQ692539	HQ692476
DO2 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Murrumbateman, New South Wales	W.M. Pitt			HQ692541	HQ692467
DO4 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Murrumbateman, New South Wales	W.M. Pitt			HQ692542	HQ692464
DO6 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Murrumbateman, New South Wales	W.M. Pitt			HQ692554	HQ692465
KC6 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Book Book, New South Wales	W.M. Pitt			HQ692557	HQ692466
SH20 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Murrumbateman, New South Wales	F.P. Trouillas			HQ692556	HQ692460
VR4 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Canowindra, New South Wales	F.P. Trouillas			HQ692544	HQ692462
CV9 ^a	<i>C. ampelina</i>	<i>Vitis vinifera</i>	Orange, New South Wales	F.P. Trouillas			HQ692545	HQ692477
WA07CO	<i>Cryptovalsa rabenhorstii</i>	<i>Vitis vinifera</i>	Cowaramup, Western Australia	F.P. Trouillas	CBS128338	DAR81041	HQ692620	HQ692522
WA08CB	<i>C. rabenhorstii</i>	<i>Vitis vinifera</i>	Cowaramup, Western Australia	F.P. Trouillas	CBS128339	DAR81042	HQ692619	HQ692523
DSORB100	<i>C. rabenhorstii</i>	<i>Sambucus nigra</i>	Mendocino Co., CA, USA	F.P. Trouillas			HQ692621	
DSORB300	<i>C. rabenhorstii</i>	<i>Sambucus nigra</i>	Mendocino Co., CA, USA	F.P. Trouillas			HQ692622	
CG14 ^a	<i>Diatrype</i> sp.	<i>Vitis vinifera</i>	Tumbarumba, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692538	HQ692507
CNP01	<i>Diatrype brunneospora</i>	<i>Acacia longifolia</i> subsp. <i>sophorae</i>	Coorong, South Australia	F.P. Trouillas		DAR80711	HM581946	HQ692478
HVGRF03	<i>Diatrypella vulgaris</i>	<i>Citrus paradisi</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128327	DAR81030	HQ692590	HQ692502
HVFRA02	<i>D. vulgaris</i>	<i>Fraxinus angustifolia</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692591	HQ692503
HVFRA04	<i>D. vulgaris</i>	<i>Fraxinus angustifolia</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128328	DAR81031	HQ692592	
HVPT01	<i>D. vulgaris</i>	<i>Schinus molle</i> var. <i>areira</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128329	DAR81032	HQ692594	HQ692506
CG7 ^a	<i>D. vulgaris</i>	<i>Vitis vinifera</i>	Tumbarumba, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692593	HQ692504
CG8 ^a	<i>D. vulgaris</i>	<i>Vitis vinifera</i>	Tumbarumba, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692595	HQ692505
ADSC300	<i>Eutypa lata</i>	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, South Australia	F.P. Trouillas			HQ692610	HQ692493

Table 1 (continued)

Collection number	Species	Host	Origin	Collector/Isolator	CBS accession no.	DAR accession no.	ITS rDNA GenBank accession no.	β -tubulin GenBank accession no.
ADSC400	<i>E. lata</i>	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, South Australia	F.P. Trouillas			HQ692613	HQ692494
SACEA01	<i>E. lata</i>	<i>Ceanothus</i> sp..	Adelaide, South Australia	F.P. Trouillas			HQ692615	HQ692499
RGA01	<i>E. lata</i>	<i>Fraxinus angustifolia</i>	Adelaide Hills, South Australia	F.P. Trouillas			HQ692614	HQ692497
RGA03	<i>E. lata</i>	<i>Fraxinus angustifolia</i>	Adelaide Hills, South Australia	F.P. Trouillas			HQ692617	HQ692498
SAPN01	<i>E. lata</i>	<i>Populus nigra</i> 'italica'	McLaren Flat., South Australia	F.P. Trouillas			HQ692616	HQ692500
POP1 ^a	<i>E. lata</i>	<i>Populus nigra</i> 'italica'	Adelaide Hills, South Australia	F.P. Trouillas			HQ692609	HQ692496
EP18 ^a	<i>E. lata</i>	<i>Vitis vinifera</i>	Tumbarumba, New South Wales	W.M. Pitt			HQ692611	HQ692501
AHILLS	<i>E. lata</i>	<i>Vitis vinifera</i>	Adelaide Hills, South Australia	M.R. Sosnowski/A. Loschiavo			HQ692612	HQ692495
ADFIC100	<i>Eutypa leptoplaca</i>	<i>Ficus macrophylla</i>	Adelaide, South Australia	F.P. Trouillas			HQ692608	HQ692485
RGA02	<i>E. leptoplaca</i>	<i>Fraxinus angustifolia</i>	Adelaide Hills, South Australia	F.P. Trouillas			HQ692602	HQ692483
RGA04	<i>E. leptoplaca</i>	<i>Fraxinus angustifolia</i>	Adelaide Hills, South Australia	F.P. Trouillas			HQ692600	HQ692484
ABA200	<i>E. leptoplaca</i>	<i>Fraxinus angustifolia</i>	Barossa Valley, South Australia	F.P. Trouillas			HQ692601	HQ692480
ABA300	<i>E. leptoplaca</i>	<i>Fraxinus angustifolia</i>	Barossa Valley, South Australia	F.P. Trouillas			HQ692604	HQ692481
SAPA01	<i>E. leptoplaca</i>	<i>Populus alba</i>	Adelaide, South Australia	F.P. Trouillas			HQ692599	HQ692488
ADSC500	<i>E. leptoplaca</i>	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, South Australia	F.P. Trouillas			HQ692603	HQ692482
SAPN02	<i>E. leptoplaca</i>	<i>Populus nigra</i> 'italica'	McLaren Flat, South Australia	F.P. Trouillas			HQ692606	HQ692489
SAPN04	<i>E. leptoplaca</i>	<i>Populus nigra</i> 'italica'	McLaren Flat, South Australia	F.P. Trouillas			HQ692605	HQ692490
SACOO1	<i>E. leptoplaca</i>	<i>Populus nigra</i> 'italica'	Coonawarra, South Australia	F.P. Trouillas			HQ692596	HQ692486
SACOO2	<i>E. leptoplaca</i>	<i>Populus nigra</i> 'italica'	Coonawarra, South Australia	F.P. Trouillas			HQ692597	HQ692487
TUQU01	<i>E. leptoplaca</i>	<i>Quercus</i> sp.	Tumbarumba, New South Wales	F.P. Trouillas			HQ692598	HQ692491
TUPN02	<i>E. leptoplaca</i>	<i>Populus nigra</i> 'italica'	Tumbarumba, New South Wales	F.P. Trouillas			HQ692607	HQ692492
CNP03	<i>Eutypella australiensis</i>	<i>Acacia longifolia</i> subsp. <i>sophorae</i>	Coorong, South Australia	F.P. Trouillas		DAR80712	HM581945	HQ692479
ADEL100	<i>Eutypella citricola</i>	<i>Ulmus procera</i>	Adelaide, South Australia	F.P. Trouillas			HQ692580	HQ692520
ADSC100	<i>E. citricola</i>	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, South Australia	F.P. Trouillas			HQ692577	HQ692510
T10R4S7 ^a	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692578	
T2R3S3 ^a	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692575	
T3R2S2 ^a	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692576	HQ692519
HVIT03	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692582	HQ692511
HVIT07	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128330	DAR81033	HQ692579	HQ692512
HVIT08	<i>E. citricola</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692583	HQ692513
HVOT01	<i>E. citricola</i>	<i>Citrus sinensis</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128331	DAR81034	HQ692581	HQ692509
HVGRF01	<i>E. citricola</i>	<i>Citrus paradisi</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128334	DAR81037	HQ692589	HQ692521
WA02BO	<i>E. citricola</i>	<i>Vitis vinifera</i>	Western Australia	F.P. Trouillas			HQ692584	HQ692514
WA03LE	<i>E. citricola</i>	<i>Citrus limon</i>	Swan Valley, Western Australia	F.P. Trouillas			HQ692585	HQ692515
WA04LE	<i>E. citricola</i>	<i>Citrus limon</i>	Swan Valley, Western Australia	F.P. Trouillas	CBS128332	DAR81035	HQ692586	HQ692516
WA05SV	<i>E. citricola</i>	<i>Vitis vinifera</i>	Swan Valley, Western Australia	F.P. Trouillas	CBS128333	DAR81036	HQ692587	HQ692517

Table 1 (continued)

Collection number	Species	Host	Origin	Collector/Isolator	CBS accession no.	DAR accession no.	ITS rDNA GenBank accession no.	β -tubulin GenBank accession no.
WA06FH	<i>E. citricola</i>	<i>Vitis vinifera</i>	Western Australia	F.P. Trouillas			HQ692588	HQ692518
HVFIG02	<i>Eutypella cryptovalsoidea</i>	<i>Ficus carica</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128335	DAR81038	HQ692573	HQ692524
HVFIG05	<i>E. cryptovalsoidea</i>	<i>Ficus carica</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt			HQ692574	HQ692525
ADEL200	<i>Eutypella microtheca</i>	<i>Ulmus procera</i>	Adelaide, South Australia	F. P. Trouillas			HQ692559	HQ692527
ADEL300	<i>E. microtheca</i>	<i>Ulmus procera</i>	Adelaide, South Australia	F. P. Trouillas			HQ692560	HQ692528
YC16 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692561	HQ692529
YC17 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692562	HQ692537
YC18 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692563	
YC23 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692564	
YC24 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692565	HQ692530
T2R2S7 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692566	HQ692532
T7R2S2 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692567	HQ692535
T10R3S9 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692568	HQ692526
T11R4S9 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692570	HQ692531
T20R4S2 ^a	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	W.M. Pitt			HQ692571	HQ692534
HVGRF02	<i>E. microtheca</i>	<i>Citrus paradisi</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128336	DAR81039	HQ692569	HQ692533
HVVIT05	<i>E. microtheca</i>	<i>Vitis vinifera</i>	Hunter Valley, New South Wales	F.P. Trouillas/W.M. Pitt	CBS128337	DAR81040	HQ692572	HQ692536

^aIsolates followed by this letter were isolated from canker, isolates not followed by this letter were isolated from perithecia

inconclusive regarding the evolutionary relationships of these fungi (Acero et al. 2004; Carmaran et al. 2006; Trouillas et al. 2010a, b).

In Australia, little work has been conducted to investigate the diversity and taxonomy of diatrypaceous fungi. Most studies have focused on the apricot and grapevine pathogen *E. lata*, which is widespread across South Australian (SA) vineyards (Carter 1991; Highet and Wicks 1998; Lardner et al. 2005; Sosnowski et al. 2007). However, a number of additional species were documented more recently. In 2004, Mostert et al. (2004) accounted for the occurrence of *C. ampelina* from vineyards both in the Coonawarra and Eden Valley regions of SA and went on demonstrate that this fungus could cause disease in grapevine shoots. Additional Diatrypaceae were also reported from surveys of fungi associated with canker diseases in grapevine in New South Wales (NSW), but identification of these isolates remained incomplete (Pitt et al. 2010).

Diatrypaceous fungi from native plant species have been reported sporadically in Australia. In his handbook of ‘‘Australian fungi’’, Cooke (1892) described seven putative species of Diatrypaceae, including *Diatrype glomeraria* Berk., *Diatrype stigma*, *Diatrype chlorosarca* Berk. &

Broome, *Cryptovalsa elevata* Berk., *E. lata*, *E. lubidunda* (Sacc.) Thum. (= *E. leprosa* [Pers.] Berl.), and *Eutypella stellulata* (Fr. : Fr.) Sacc. Additional species were described from intertidal host plants in north Queensland, including *Cryptovalsa halosarceicola* K.D. Hyde on *Halosarcia halocnemoides* (Nees) Paul G. Wilson in a mangrove at Cairns Airport (Hyde 1993), *Eutypa bathurstensis* K.D. Hyde & Rappaz (Hyde and Rappaz 1993) and *Eutypella naqsii* K.D. Hyde (Hyde 1995) on *Avicennia* sp. at Bathurst Heads. Later, Yuan (1996) documented *Cryptovalsa protracta* (Pers.) De Not., *Diatrype stigma* and *Eutypella scoparia* (Schwein. : Fr.) Ellis & Everh. on *Acacia* and *Eucalyptus* plants on Melville Island in the Northern Territory, while Trouillas et al. (2010a, b) described two additional species from native *Acacia* shrubs in the Coorong National Park, SA. To the best of our knowledge, the above references constitute the only studies that illustrate the diatrypaceous mycota in Australia.

During this study, we conducted surveys and investigated the diversity of diatrypaceous fungi associated with grapevines and other woody plants and in SA, NSW and Western Australia (WA). In many instances, fungal colonies displaying morphological characteristics typical of Diatrypaceae were isolated from diseased grapevines. Fruiting bodies typical of

Diatrypaceae were also observed from grapevines. The diversity, identity and distribution of these fungi in the main wine grape growing regions of Australia are currently unknown. Hence, much work is necessary not only in the collection and identification of the various species, but also in the determination of their pathogenicity to grapevines and role in the overall complex of grapevine canker diseases. The objectives of this study were to collect, identify and describe the diatrypaceous fungi in and near Australian vineyards, and characterize species using morphology and molecular phylogeny.

Materials and methods

Origin and deposit of isolates During spring and summer of 2008 and 2009, we obtained strains of Diatrypaceae from cankers in infected grapevine spurs, cordons or trunks, and from fruiting bodies on dead grapevines as well as dead wood of native, ornamental and cultivated plants neighboring vineyards. Viticultural regions sampled included the Barossa Valley, Adelaide Hills, McLaren Flat, and Coonawarra in SA; the Hunter Valley, the Riverina, and Southern NSW regions; and the Swan Valley, Margaret River, and Great Southern regions of WA. Isolates from the National Wine and Grape Industry Centre (Charles Sturt University, Wagga Wagga, NSW, Australia) collected in previous surveys (Pitt et al. 2010) were also used in this study. The geographic origin and host range of the specimens collected during this study are summarized in Table 1.

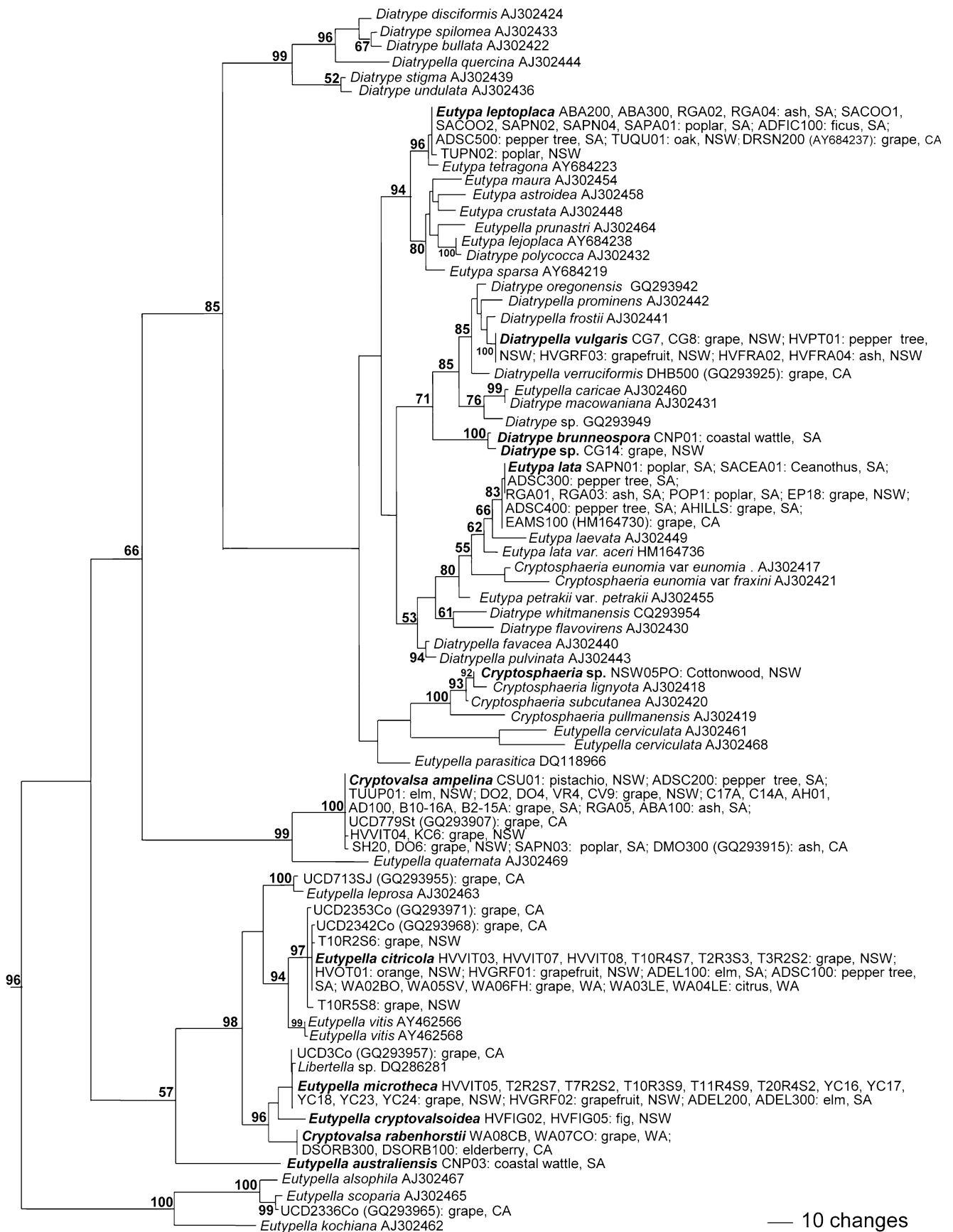
Isolates were grown from ascospores or from hyphae in infected grapevine wood as described by Trouillas et al. (2010a, b). Pure cultures were obtained by transferring single hyphal tips onto potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, Hampshire, England) amended with 100 ppm tetracycline (PDA-tet). Representative isolates, including ex-type cultures (fresh cultures) of Diatrypaceae from Australia were deposited both at Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (accession no: CBS128327- CBS128339), and at the Australian Scientific Collections (DAR), Industry & Investment NSW, Orange, NSW, Australia (accession no: DAR81030-DAR81042). Dry specimens (bark and/or wood) containing the perfect stage of each fungal isolate were also deposited at DAR.

Identification and morphological analysis Fruiting bodies of Diatrypaceae were identified in conformity with the treatments of Glawe and Rogers (1984) and Rappaz (1987). In addition, putative new species of *Eutypella*, *Diatrypella* and *Cryptovalsa* were compared with descriptions and illustrations in Saccardo's *Sylloge Fungorum* vol. 1 (1882), Ellis and Everharts (1892), and Berlese (1900) to verify

Fig. 1 One of the 36 most-parsimonious trees obtained from the ITS sequence data. (TL=1518 steps, CI=0.4302, RI=0.7444, RC=0.3202). Bootstrap support values from 1000 replicates higher than 50% are reported at the nodes. Species names in bold represent species occurring in Australia

species originality. Specimens from Australia were also compared with reference specimens from California (Trouillas et al. 2010a, b) using morphological and phylogenetic analyses. Microscopic examinations were carried out with standard light microscopy on an Olympus Provis AX70TRF (Olympus Optical Co. Ltd., Japan) microscope fitted with a ColorView IIIu digital camera (Soft Imaging Systems (SIS) GmbH, Munster, Germany). Conidial masses as well as perithecial contents were mounted in water and observed by brightfield microscopy. Digital images were recorded using analySIS LS Research 2.41 (SIS) and measurements for at least 30 conidia, 50 ascospores and 20 asci were made for each specimen. To study colony morphology and conidial production, cultures on PDA were maintained in incubators under controlled conditions of intermittent fluorescent lighting (12 h) at 24°C.

DNA isolation, amplification and phylogenetic analyses DNA extractions were performed as described by Pitt et al. (2010). Total genomic DNA was extracted from pure cultures after transferring colonized agar plugs into 50 mL Falcon tubes filled with 20 mL of potato dextrose broth (Oxoid Ltd., Basingstoke, Hampshire, England). Broth cultures were then incubated on a Sartorius Certomat BS-1 (Goettingen, Germany) orbital shaker revolving at 90 rpm for 7 days at 25°C. Mycelia were collected by filtration, lyophilized and DNA was extracted using the Qiagen Plant Mini Kit according to the manufacturer's instructions (Qiagen Pty Ltd, Clifton Hills, Vic., Australia). The internal transcribed spacer regions (ITS1 and ITS2), including the 5.8 S rDNA operon of the nuclear ribosomal DNA region were amplified by the polymerase chain reaction (PCR) using primers ITS5 and ITS4 (White et al. 1990). Partial sequence of the β -tubulin gene was amplified using primers Bt2a and Bt2b (Glass and Donaldson 1995). Each PCR tube contained 0.1 volume of 10 \times buffer (15 mM MgCl₂, Qiagen), 200 mM each of dNTPs, 0.15 mM of each primer, 1 unit of HotStar *Taq* DNA polymerase (Qiagen), and ~50 ng of DNA template, and were adjusted with sterile nanopure water to a total volume of 50 μ L. PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification was accomplished by an initial step of 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1.5 min at 72°C, with a final extension of 5 min at 72°C. PCR products were separated by electrophoresis on 1% agarose gels containing 0.5 \times Tris-borate-EDTA buffer. Positive amplifications were



— 10 changes

confirmed by photography under UV light following staining with ethidium bromide (0.5 mg/L). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA). Both strands of the ITS and β -tubulin regions were sequenced by the Australian Genome Research Facility (University of Queensland, St Lucia, Qld, Australia). Sequencing results were edited and assembled using Sequencher™ version 3.1.1. Sequences were aligned using ClustalW multiple alignment program (Thompson et al. 1994) and were adjusted manually using BioEdit Sequence Alignment Editor Version 7.0.8. (Hall 1999). Phylogenetic analyses were performed with PAUP version 4.0b10 (Swofford 1999) using maximum parsimony (MP) with a heuristic search and 1000 random addition sequence replicates. Tree bisection-reconnection (TBR) was used as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Ambiguously aligned regions were not excluded for phylogenetic analyses and alignment gaps were treated as missing data. Measures including tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated. Bootstrap support (BS) was calculated using 1000 replicates to test branch strength. Sequences have been deposited into GenBank (HQ692458–HQ692622). To accelerate the process, phylogenetic analyses were run using a single representative of each haplotype. Sequences of *Xylaria hypoxylon*, *Daldinia concentrica*, *Anthostomella eucalytorum*, *A. protea*, *Nemania aenea* and *Camileia tinctor* from GenBank were used as outgroup in the ITS analysis. Beta tubulin trees were rooted using *E. scoparia* as outgroup.

Results

Phylogenetic analyses ITS and β -tubulin sequences were obtained for approximately 90 isolates of Diatrypeaceae collected in Australia. Unique ITS sequences or haplotypes were aligned with approximately 50 GenBank reference sequences, while the β -tubulin dataset included 24 sequences obtained from GenBank.

The ITS analysis comprised 74 taxa and 636 characters, of which 276 were constant, 83 parsimony-uninformative and 277 parsimony-informative. The heuristic search using the ITS dataset resulted in 36 most parsimonious trees of similar topologies, each comprising 1518 steps (CI=0.4302, RI=0.7444, RC=0.3202 and HI=0.6126). One of the 36 most parsimonious (MP) trees is shown in Fig. 1.

In contrast, the β -tubulin dataset contained 45 taxa and 417 characters, of which 207 were constant, 17 parsimony-uninformative, and 194 parsimony-informative. The MP

Fig. 2 One of the 10 most-parsimonious trees obtained from the β -tubulin sequence data. (TL=703 steps, CI=0.5391, RI=0.8253, RC=0.4450). Bootstrap support values from 1000 replicates higher than 50% are reported at the nodes. Species names in bold represent species occurring in Australia

analysis resulted in 10 trees, each with a length of 703 steps (CI=0.5391, RI=0.8253, RC=0.4450 and HI=0.4723). Each most parsimonious tree shared the same overall topology, one of which is shown in Fig. 2.

Grouping of genera and species was generally similar for the ITS and β -tubulin analyses. Bootstrap values from the ITS and β -tubulin data sets (98% and 87% respectively) supported the occurrence of a main clade comprising several *Eutypella* and *Cryptovalsa*-like spp. (Figs. 1–2). *E. microtheca* (with 8-spored asci) grouped with the polyporous spp. *Eutypella cryptovalsoidea* and *C. rabenhorstii* (96% and 98% respectively) (Figs. 1–2). Similarly, the octosporous *D. oregonensis* was closely related to various polyporous *Diatrypella* spp. (85% and 96% respectively) (Figs. 1–2).

In the ITS analysis, *Diatrype spilomea*, *D. bullata*, *D. disciformis*, *D. stigma*, *D. undulata* and *Diatrypella quercina* formed another clade and demonstrated strong affinities (96% bootstrap value) (Fig. 1). *Eutypa* spp. separated into two major clades. The first clade included *E. lata*, *E. lata* var. *aceri*, *E. laevata*, *E. petrakii* var. *petrakii* and also included *C. eunomia* (80% bootstrap value). The second clade included all remaining *Eutypa* species that were tested (94% bootstrap value) and also included *E. prunastri* and *D. polycocca* (Fig. 1). Isolates NSW01PO–NSW04PO appeared to be closely related to *C. lignyota*.

Taxonomy

Descriptions are provided for novel or unusual species. Tables 2 and 3 illustrate conidial, ascus and ascospore sizes for all isolates examined in this study. Measurements under the following descriptions represent averaged sizes obtained from the different isolates.

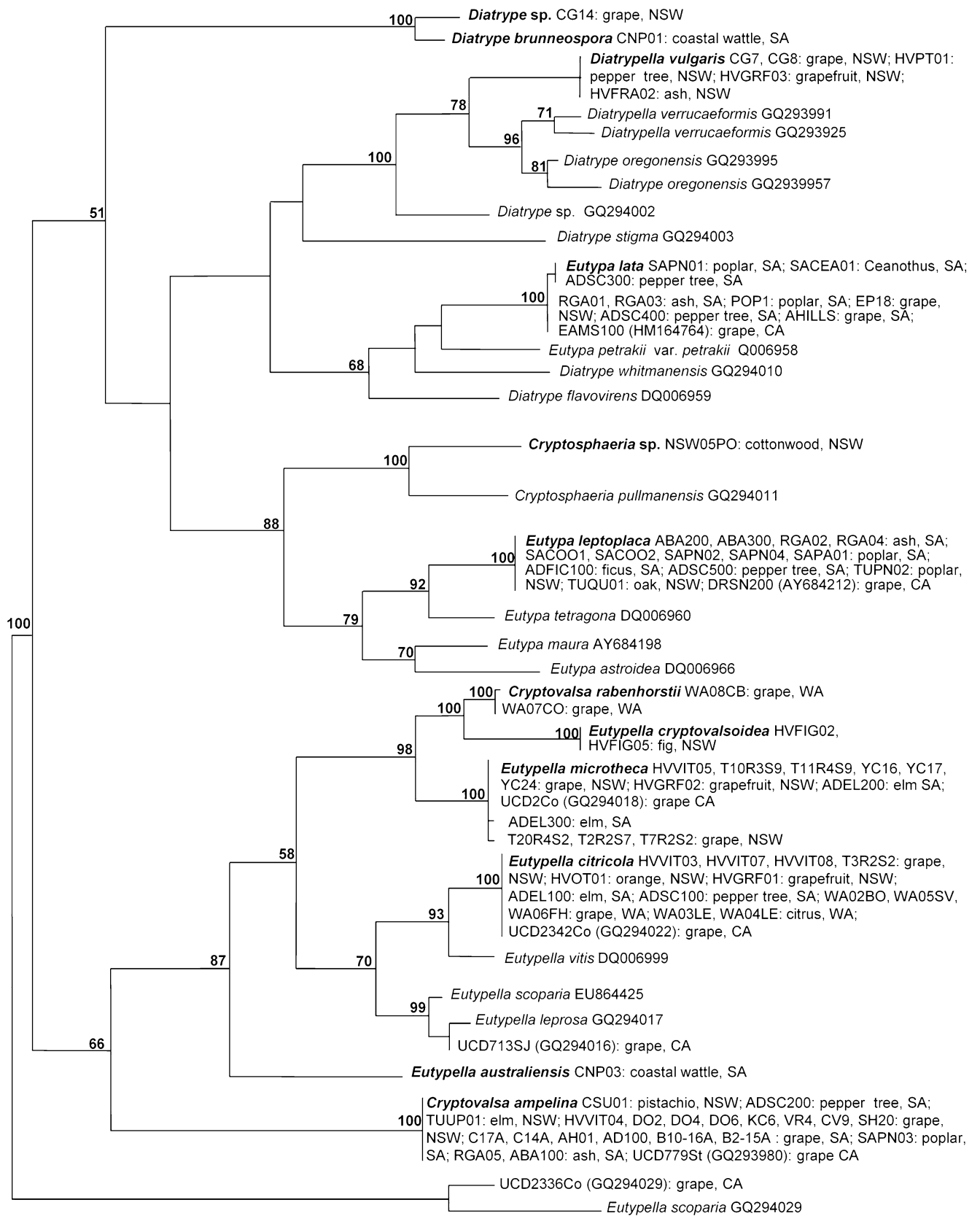
Cryptovalsa rabenhorstii (Nitschke) Sacc., Myc. Ven. 135, tab. XIV.

(Fig. 3)

Basionym: *Valsa rabenhorstii* Nitschke Pyr. Germ.

Synonym: *Sphaeria spiculosa* var. *robiniae* Rabenh., in Exsicc. Klotzsch, Herb. myc.

Stromata in bark of lignified canes (*V. vinifera*), poorly developed, perithecia buried in the inner bark and scattered in subvalsiform groups of 2–3, or fairly irregularly in larger groups, raising the epidermis which is not discolored and remains attached, or which rupture longitudinally revealing



— 5 changes

Table 2 Conidial sizes for various isolates of Diatrypaceae

Species name/Collection number	Conidia full length (μm)	Conidia chord length (μm)	Conidia width (μm)
<i>Diatrypella vulgaris</i>			
CG8	(37.18–) 46.47–49.37 (–60.10)	(24.31–) 39.51–42.07 (–49.97)	(1.56–) 2.00–2.13 (–2.56)
HVGRF03	(45.23–) 59.08–62.61 (–74.61)	(25.27–) 43.81–47.60 (–57.60)	(1.15–) 1.58–1.86 (–2.25)
HVFRA04	(40.46–) 48.14–50.58 (–60.49)	(29.07–) 39.61–41.62 (–50.07)	(1.12–) 1.39–1.52 (–1.97)
HVGRF02	(15.05–) 18.23–19.26 (–23.90)	(11.68–) 14.74–15.40 (–18.46)	(1.44–) 2.00–2.19 (–2.38)
<i>Eutypella citricola</i>			
HVOT01	(14.97–) 18.51–19.18 (–21.37)	(13.77–) 15.93–16.62 (–19.83)	(1.39–) 1.67–1.83 (–1.97)
WA02BO	(11.34–) 13.48–14.14 (–17.02)	(12.99–) 16.08–16.93 (–20.38)	(0.92–) 1.24–1.32 (–1.52)
WA03LE	(10.71–) 13.25–14.03 (–16.45)	(12.49–) 15.13–15.93 (–19.11)	(1.13–) 1.36–1.41 (–1.57)
WA04LE	(16.00–) 21.31–23.13 (–32.37)	(24.96–) 31.15–33.46 (–47.19)	(1.00–) 1.25–1.30 (–1.48)
WA05SV	(17.03–) 20.00–21.17 (–29.74)	(18.98–) 26.38–28.18 (–39.39)	(1.10–) 1.29–1.35 (–1.56)
WA06FH	(11.28–) 14.04–15.03 (–17.95)	(12.53–) 15.48–16.44 (–20.13)	(0.97–) 1.18–1.23 (–1.41)
WA09LE	(11.44–) 13.23–13.92 (–16.57)	(13.13–) 16.31–17.20 (–20.54)	(1.06–) 1.25–1.30 (–1.49)
<i>Eutypella microtheca</i>			
HVVIT05	(15.64–) 20.76–21.77 (–25.50)	(15.78–) 18.41–19.25 (–22.43)	(1.31–) 1.58–1.73 (–1.91)
HVVIT07	(15.32–) 19.21–20.34 (–23.66)	(12.54–) 16.74–17.60 (–20.44)	(1.48–) 1.69–1.82 (–2.10)
HVVIT08	(12.80–) 18.11–19.19 (–23.13)	(13.92–) 16.81–17.55 (–21.09)	(1.33–) 1.45–1.60 (–1.91)
YC18	(16.38–) 20.91–21.86 (–25.20)	(14.00–) 17.63–18.82 (–23.79)	(1.33–) 1.45–1.52 (–1.64)

groups of black ostioles occasionally sheltered around a white ectostroma, which apparently facilitate pressuring and splitting of the bark; perithecia outer surface coated with white, powdery entostroma, 0.35–0.55 mm diam, ostioles poorly emerging, more or less distinctly quadrisulcate. *Asci* long-pedicellate, polysporous, p. sp. (55–)70–90 (–95)×(15–)18–22(–27) μm . *Ascospores* hyaline when immature turning yellowish to light-brown at maturity, sub-allantoid, cylindrical to oblong, (10–)13.5–15 (–17.5)×(3.2–)4–5(–6) μm . *Colonies* white with rather irregular margin. *Conidia* not seen.

Hosts. *Vitis vinifera* (Australia, WA), *Sambucus nigra* (USA, CA).

Notes This species has characteristics typical of members of the genus *Cryptovalsa*, and resembles closely descriptions of *C. rabenhorstii* (Nitschke 1867; Saccardo 1882) as well as the illustration by Berlese (1900) of *C. ampelina*, *C. rabenhorstii* var. *rosarum* and *C. rabenhorstii* var. *eutypelloidea*. However, as we could not find the type specimen nor obtain culture collections for this species, identification remains tentative. Also, phylogenetic analyses show affinities of this fungus with *Eutypella* spp. The assignment of this isolate to the genus *Cryptovalsa* may therefore require future reconsideration. Hence, it is preferable not to propose a novel combination for this species until identification of types and further large scale phylogenetic studies of the Diatrypaceae can be conducted.

Specimens examined. AUSTRALIA, WA, Great Southern regions, on lignified canes of *Vitis vinifera* on the ground,

Nov. 2009, F. P. Trouillas, coll. number WA07CO, DAR81041, CBS128338; and coll. number WA08CB, DAR81042, CBS128339.

Diatrypella vulgaris Trouillas, W. M. Pitt & Gubler, **sp. nov.**

(Fig. 4)

Mycobank: MB 519404

Etymology Vulgaris, meaning ordinary, to account for the typical *Diatrypella* morphology of this fungus.

Stromata erumpentia, in pustulis 1–4 μm longis, saepe a nigro lineamento in infero ligno evidente circumscripta, per corticem vel lignum dehiscencia atque a reliqua adhaerente cute vel ligneis fragmentis saepe circumfusa, incomposita et congruente vel hemispherica atque iuxta ligneis striis oblonga formis variantia. Perithecia circinata vel ovoidea, aliquando compressa, ex albo entostroma amplexa, 0.25–0.45 mm diametro. Ostiola sulcata, parum eminentia. Asci brevioribus caulis, paraphysati, polyspori, parte sporifera (65–)80–130 (–155)×(12–)18–20 μm . Ascospores allantoidae, corpore flavidae (7–)8–10(–12)×2–2.5 μm . Albae coloniae leviter fuscae aetate se vertentes, una specie cum subexcelso mycelio pycnidia constituenta, conidia ad parum lutea corpora manantia. Conidia fili instar, 25–40(–55)×(1–)1.5–2 μm .

Stromata well developed, in pustules 1–4 mm in length, often delimited with a black line perceptible in the wood below, bursting through bark or wood and often surrounded by remaining adherent epidermis or wood fragments, varying in shape from irregular and confluent to hemispherical and oblong following wood striations,

Table 3 Ascus and ascospore sizes for various isolates of Diatrypaceae

Species name/ Collection number	Ascospore length (µm)	Ascospore width (µm)	Ascus length (µm)	Ascus width (µm)
<i>Cryptosphaeria</i> sp.				
NSW02PO	(8.80–) 11.47–12.14 (–13.90)	(2.53–) 3.15–3.37 (–4.49)	(42.27–) 50.13–56.47 (–63.45)	(6.50) 10.53–12.45 (–13.87)
NSW04PO	(11.35–) 13.05–13.46 (–14.83)	(2.41–) 3.02–3.24 (–3.72)	(42.21–) 52.48–57.96 (–71.61)	(7.43–) 9.04–9.77 (–11.08)
<i>Cryptovalsa ampelina</i>				
CSU01	(9.83–) 11.24–11.65 (–13.78)	(2.15–) 2.76–2.91 (–3.57)	(73.11–) 86.06–129.02 (–135.80)	(11.41–) 11.43–13.27 (–14.47)
HVVIT04	(7.97–) 10.02–10.49 (–12.87)	(2.19–) 2.90–3.06 (–3.85)	(62.13–) 80.14–90.94 (–112.56)	(11.11–) 12.47–13.92 (–17.03)
<i>Cryptovalsa rabenhorstii</i>				
WA07CO	(12.74–) 14.43–14.95 (–17.50)	(3.22–) 3.80–3.96 (–4.53)	(65.82–) 77.30–88.47 (–95.34)	(15.43–) 18.63–22.62 (–27.70)
WA08CB	(10.29–) 13.44–14.38 (–17.60)	(3.61–) 4.59–4.86 (–6.04)	(54.05–) 66.84–75.54 (–92.46)	(15.01–) 17.64–18.83 (–21.55)
<i>Diatrypella vulgaris</i>				
HVPT01	(7.23–) 8.75–9.11 (–11.26)	(1.61–) 2.31–2.44 (–3.20)	(83.45–) 99.22–111.03 (–122.42)	(11.89–) 13.57–14.90 (–16.72)
HVFR04	(7.16–) 8.83–9.14 (–10.42)	(1.71–) 2.36–2.48 (–3.00)	(69.11–) 87.08–97.53 (–119.74)	(15.49–) 18.25–19.79 (–22.34)
HVGRF03	(7.10–) 8.69–9.25 (–12.04)	(1.89–) 2.29–2.42 (–2.91)	(82.37–) 104.16–120.63 (–152.22)	(9.76–) 13.17–15.11 (–19.83)
<i>Eutypa leptoplaca</i>				
TUQU01	(6.59–) 8.35–8.65 (–9.64)	(1.84–) 2.51–2.71 (–3.67)	(24.38–) 29.55–32.22 (–37.66)	(6.30–) 7.07–7.55 (–8.35)
TUPN02	(5.92–) 7.55–7.80 (–9.01)	(1.64–) 2.14–2.26 (–2.82)	(29.96–) 33.36–37.31 (–47.24)	(5.26–) 6.63–7.41 (–9.16)
<i>Eutypella citricola</i>				
HVVIT07	(7.78–) 9.73–10.24 (–12.03)	(2.02–) 2.20–2.34 (–2.71)	(37.36–) 46.13–50.77 (–60.83)	(6.39–) 7.23–7.79 (–9.61)
HVVIT08	(6.95–) 9.46–9.91 (–11.81)	(1.73–) 2.14–2.26 (–2.51)	(41.45–) 46.16–49.34 (–56.26)	(6.39–) 7.20–7.62 (–8.77)
HVOT01	(7.84–) 9.17–9.60 (–11.07)	(2.03–) 2.50–2.71 (–3.12)	(32.83–) 38.89–44.71 (–51.65)	(6.45–) 7.18–8.01 (–8.81)
ADEL100	(7.24–) 8.01–8.28 (–9.30)	(1.36–) 1.82–1.94 (–2.38)	(37.05–) 43.25–46.34 (–51.47)	(5.65–) 6.82–7.81 (–12.50)
HVGRF01	(8.07–) 9.30–9.73 (–12.30)	(1.91–) 2.14–2.33 (–2.60)	(39.40–) 42.07–45.52 (–50.27)	(7.49–) 7.58–7.79 (–7.93)
WA01SV	(9.96–) 11.51–11.98 (–13.94)	(2.20–) 2.73–2.90 (–3.59)	(37.93–) 51.81–60.91 (–70.08)	(7.66–) 8.94–10.08 (–12.35)
WA02BO	(7.96–) 9.21–9.62 (–11.13)	(1.88–) 2.18–2.30 (–2.51)	(35.21–) 41.27–45.02 (–58.39)	(7.13–) 8.01–8.51 (–9.36)
WA03LE	(6.91–) 9.13–9.59 (–11.22)	(2.14–) 2.39–2.51 (–2.75)	(34.15–) 40.13–42.55 (–48.46)	(6.89–) 8.04–8.52 (–9.34)
WA04LE	(7.71–) 9.38–9.83 (–12.31)	(1.94–) 2.25–2.38 (–2.74)	(34.07–) 40.39–44.67 (–52.39)	(6.84–) 7.71–8.29 (–9.24)
WA05SV	(7.95–) 9.25–9.64 (–10.80)	(2.00–) 2.27–2.37 (–2.59)	(37.00–) 45.73–48.96 (–53.59)	(7.33–) 8.19–8.85 (–9.75)
WA06FH	(9.69–) 11.45–11.92 (–13.68)	(2.06–) 2.52–2.65 (–3.01)	(41.70–) 49.27–56.42 (–64.33)	(8.24–) 9.19–9.77 (–10.82)
WA65SV	(9.02–) 10.18–10.56 (–12.62)	(1.97–) 2.60–2.75 (–3.35)	(31.70–) 44.65–52.44 (–63.21)	(7.59–) 8.95–9.99 (–11.47)
WA09LE	(8.89–) 11.50–12.12 (–13.97)	(2.47–) 3.06–3.20 (–3.89)	(41.57–) 47.64–53.44 (–61.40)	(7.10–) 8.45–9.21 (–10.34)
<i>Eutypella cryptovalsoidea</i>				
HVFIG01	(9.03–) 11.09–11.49 (–13.39)	(2.71–) 3.19–3.34 (–3.91)	(62.83–) 91.26–102.39 (–118.47)	(15.34–) 17.79–19.12 (–20.94)
HVFIG02	8–10	2.5–3	60–100	(11–) 15–18 (–35)
<i>Eutypella microtheca</i>				
ADEL300	(7.99–) 9.44–9.87 (–11.28)	(1.72–) 2.08–2.17 (–2.59)	(35.60–) 41.55–46.65 (–54.22)	(7.27–) 8.07–8.59 (–9.19)
HVGRF02	(6.63–) 8.65–9.10 (–10.65)	(1.85–) 2.08–2.19 (–2.46)	(35.86–) 43.99–49.66 (–61.58)	(6.58–) 7.83–8.39 (–9.00)
HVVIT05	(8.19–) 9.39–9.76 (–10.95)	(2.00–) 2.29–2.44 (–2.62)	(32.24–) 37.03–42.51 (–49.63)	(7.23–) 7.65–8.75 (–9.94)

perithecia circular to ovoid, occasionally compressed, surrounded by white entostroma, 0.25–0.45 mm diam, ostioles sulcate, only slightly prominent. *Asci* with moderately short stalks, paraphysate, polysporous, p. sp. (65–)80–130(–155)×(12–)18–20 µm. *Ascospores* allantoid, yellowish in mass (7–)8–10(–12)×2–2.5 µm. *Colonies* white becoming light brown with age, homogeneous with rather moderate aerial mycelium, forming pycnidia

exuding conidia in light orange masses. *Conidia* filiform, 25–40(–55)×(1–)1.5–2 µm.

Hosts. *Citrus paradisi*, *Fraxinus angustifolia*, *Schinus molle* var. *areira* (Australia, NSW).

Notes. This fungus shows morphological characteristics typical of fungi in the genus *Diatrypella* and resembles in many aspects earlier descriptions of *D. verruciformis* and *D. pulvinata*. However, this species

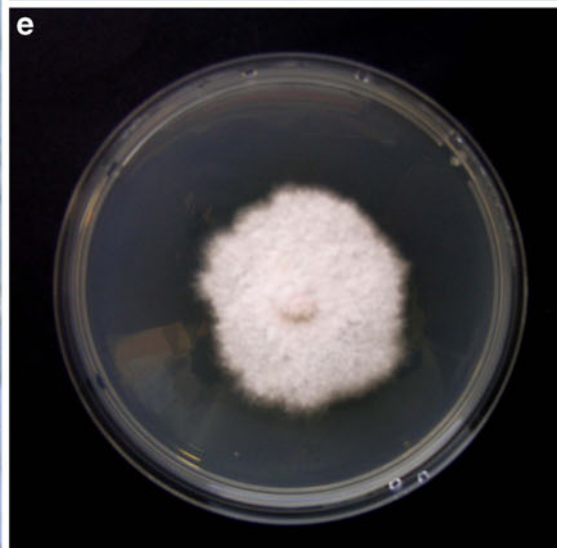


Fig. 3 Morphology of *Cryptovalsa rabenhorstii*. **a.** Perithecial stroma in the bark of a lignified cane of *Vitis vinifera*; **b.** Emerging perithecial ostioles surrounded with white ectostroma and perithecial cavities; **c.** Long-pedicellate polysporous ascus; **d.** Mature (light brown) and immature (hyaline) ascospores; **e.** Colony after 29 days on 85 mm diam PDA dish incubated under intermittent fluorescent lighting (12 h). Bars=1 cm in a; 1 mm in b; 50 μ m in c–d

can be distinguished on characteristics of the asci which are longer and unusually wide, and which bear longer ascospores than most previously described species (commonly 6–8 μ m) (Saccardo 1882; Ellis and Everharts 1892; Berlese 1900; Glawe and Rogers 1984). Also, ITS sequences of this fungus differed from all *Diatrypella* spp. sequences available in GenBank, including *D. pulvinata* and *D. verruciformis*.

Specimens examined. AUSTRALIA, NSW, Hunter Valley, on dead branches of *Citrus paradisi*, Dec. 2008, HOLOTYPE: F. P. Trouillas & W. M. Pitt, coll. number HVGRF03, DAR81030, CBS128327; on dead branches of *Fraxinus angustifolia*, Dec. 2008, ISOTYPE: F. P. Trouillas & W. M. Pitt, coll. number HVFRA04, DAR81031, CBS128328; on dead branches of *Schinus molle* var. *areira*, Dec. 2008, ISOTYPE: F. P. Trouillas & W. M. Pitt, coll. number HVPT01, DAR81032, CBS128329.

Eutypella citricola Speg., Anales del Museo Nacional de Buenos Aires 6: 245, 1898. (Fig. 5)

Stromata in the bark or wood, which appear rugous, in pustules scattered or aggregated into large surface, pustules often delineated with black line; *perithecia* surrounded by white, powdery entostroma, attached onto the outer surface, circular to ovoid, sometimes compressed with others, 0.25–0.5 mm diam; ostioles raising and piercing the periderm; *ostioles* slightly emerging through the periderm, in contact within small groups, well define, 3–4 sulcate. *Asci* 8-spored, clavate, p. sp. 55–80 \times 7.5–9 μ m. *Ascospores* allantoid, subhyaline to light yellow, (9–)10.5–12(–13) \times 2–3 μ m. *Colonies* white and even, moderate aerial mycelium, forming numerous dots of melanized mycelium spread across the media and visible from the underside after 30 days incubation on PDA at 24°C. *Conidia* filiform 15–20(–25) \times 1.5–2 μ m.

Hosts *Citrus limon*, *Citrus sinensis*, *Citrus paradisi* (Australia, NSW); *Schinus molle* var. *areira*, *Ulmus procera* (Australia, SA); *Vitis vinifera* (Australia, NSW; USA, CA).

Notes Collections from Australia were morphologically identical to the type specimen of *Eutypella citricola* and the identification of these isolates is confident.

Specimens examined ARGENTINA, La Trinidad, prov. Tucumán, on branch of *Citrus aurantium*, Jan. 1895, HOLOTYPE: Speg., LPS-2120. AUSTRALIA, NSW, Hunter Valley, on dead branches of *Vitis vinifera*, Dec.

2008, F. P. Trouillas & W. M. Pitt, coll. number HVVIT07, DAR81033, CBS128330; on dead branches of *Citrus sinensis*, Dec. 2008, F. P. Trouillas & W. M. Pitt, coll. number HVOT01, DAR81034, CBS128331; on dead branches of *Citrus paradisi*, Dec. 2008, F. P. Trouillas & W. M. Pitt, coll. number HVGRF01, DAR81037, CBS128334; WA, Swan Valley, on dead branches of *Citrus limon*, Nov. 2009, F. P. Trouillas, coll. number WA04LE, DAR81035, CBS128332; on dead branches of *Vitis vinifera*, Nov. 2009, F. P. Trouillas, coll. number WA05SV, DAR81036, CBS128333; SA, Adelaide, Waite Campus, on dead branches of *Ulmus procera*, Nov. 2008, F. P. Trouillas, coll. number ADEL100; on dead branches of *Schinus molle* var. *areira*, Nov. 2008, F. P. Trouillas, coll. number ADSC100.

Eutypella cryptovalsoidea Trouillas, W. M. Pitt & Gubler, **sp. nov.**

(Fig. 6)

Mycobank: MB 519406

Etymology. *Cryptovalsoidea*, referring to the morphological similitude of this fungus with *Cryptovalsa*.

Stromata plerumque in cortice, male evoluta circa fundum perithecialem, nigra, effusa atque paulo callosiora circa cervices peritheciales sub peridermio. Perithecia plus minus inter se coniuncta et ad copiosos coetus congruentia, inaequaliter constratos. Ostiola hemisphaerica, saepe perforata, singula vel coniunctim per corticem eminentia. Asci clavati vel fusiformes, longe pedicellati, polyspori, parte sporifera 65–120 \times 15–20 μ m. Ascospores flavidae, in corpore aquiliorae, allantoideae vel sub-allantioideae, 8–12(–13.5) \times 2–3 μ m. Coloniae albae cum subexcelso mycelio tenuique areo-roseo inferiore. Conidia non evidentia.

Stromata mostly in bark, poorly developed around the perithecial base, black, effuse and rather crusty around perithecial necks below the periderm; *perithecia* more or less in contact and confluent into large groups, irregularly scattered; *ostioles* hemispherical, often perforated, emerging singly or in groups through bark. *Asci* clavate to spindle-shape, long-pedicellate, polysporous, p. sp. 65–120 \times 15–20 μ m. *Ascospores* yellowish, darker in mass, allantoid to sub-allantoid, 8–12(–13.5) \times 2–3 μ m. *Colonies* white with rather moderate aerial mycelium and slight orange-pink underside. *Conidia* not seen.

Hosts. *Ficus carica* (Australia, NSW).

Notes. The present species displays some features of morphology typical of *Cryptovalsa* (poorly developed stroma, polysporous ascus) as well as *Eutypella* (perithecial necks erumpent in groups). Because of the polyporous ascus, this species could be referred as *Cryptovalsa* under the current classification scheme for Diatrypaceae. However, size and shape of the polysporous asci differed from all *Cryptovalsa* species previously described from

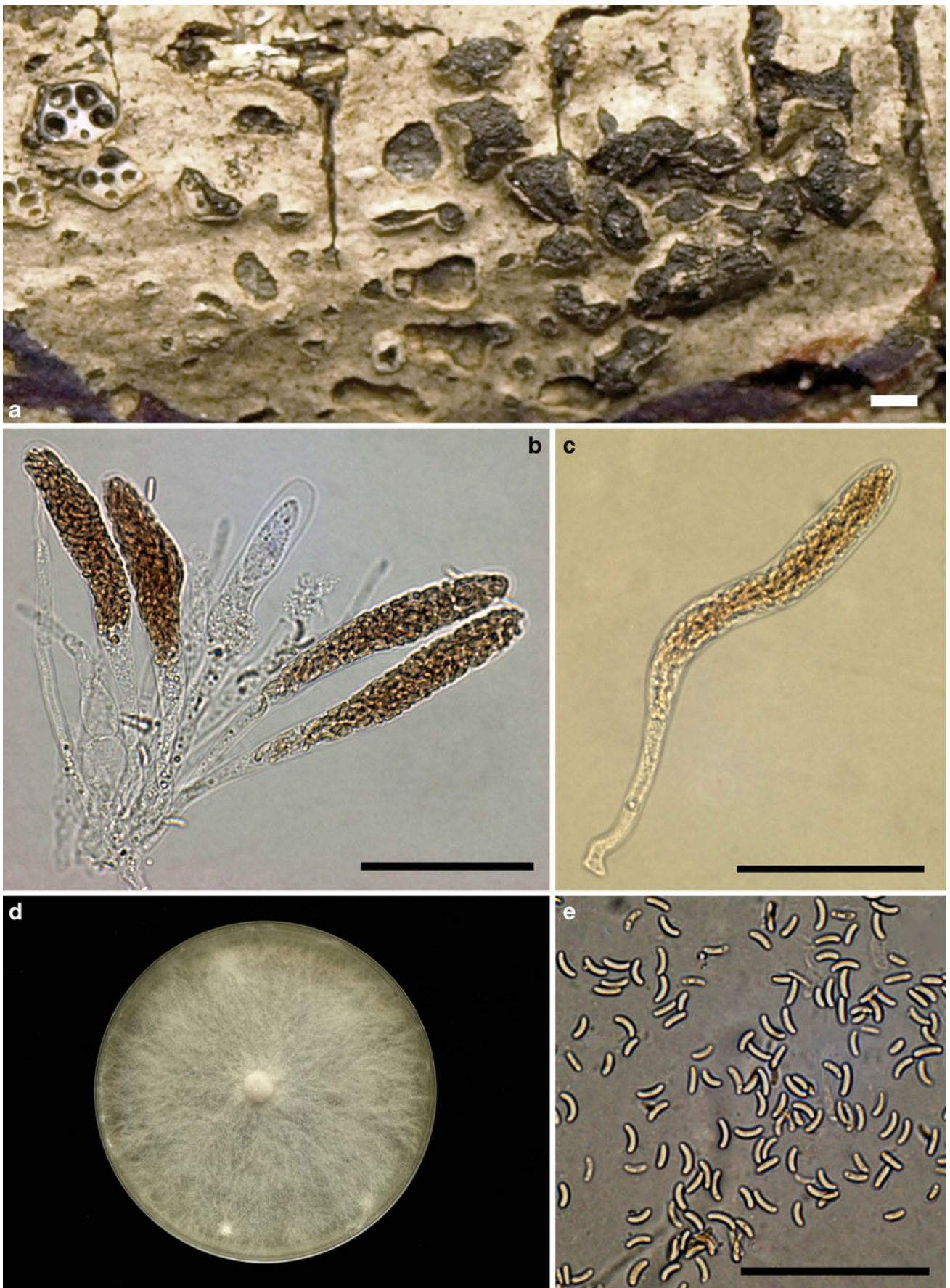


Fig. 4 Morphology of *Diatrypella vulgaris*. **a.** Pustulate stromata with white entostroma embedded in the bark of *Fraxinus angustifolia*; **b.** Group of polysporous asci and paraphyses; **c.** Close up on the rather short-stalked ascus, with wide and lengthy spore-bearing portion; **d.** Colony after one month incubation in the dark at 25°C on 85 mm PDA dish; **e.** Allantoid ascospores. Bars=1 mm in a; 50 µm in b–c; 50 µm in e

Ficus carica and additional host plants. (Saccardo 1882; 1905; 1926; Berlese 1900; Spooner 1981).

Specimens examined. AUSTRALIA, NSW, Hunter Valley, on dead branches of *Ficus carica*, Dec. 2008, HOLOTYPE: F. P. Trouillas & W. M. Pitt, coll. number HVFIG02, DAR81038, CBS128335.

Eutypella microtheca Trouillas, W. M. Pitt & Gubler, **sp. nov.**

(Fig. 7)

Mycobank: MB 519407

Etymology. Microtheca, referring to the small diam of the perithecia.

Stromata in cortice: subrigentia extremitatem peridermii (tumens aspectus), quod ex eminentibus, non-proiectis ostioliis laceratur; stromata in ligno: paulo magis eutypeia, ligni superficiem fuscantia tollentiaque. Perithecia plus minusve inter se coniuncta, globosa vel ovoidea, a candida, pulverulenta trama circumfusa, minuta, 0.15–0.25(–0.3) µm diametro; ostiola 3–4 sulcata. Asci octospori, clavati, longe stipitati, parte sporifera 35–55(–60) × 7–9 µm. Ascosporeae allantoidae, subhyalinae vel flavescens, 8–10(–11) × 2–2.5 µm. Coloniae roseae, ad canum vergentes et crebra pycnidia conficientes. Conidia fili instar 16–22(–25) × 1.5–2 µm.

Stromata in bark: elevating the periderm surface (swollen appearance), which become ripped off by the emerging, non-prominent ostioles; stromata in wood: rather eutypoid, blackening and raising the wood surface. *Perithecia* more or less in contact, round to ovoid, surrounded by white, powdery entostromatic tissue, minute, 0.15–0.25(–0.3) mm diam; *ostioles* 3–4 sulcate. *Asci* 8-spored, clavate, long-stipitate, p. sp. 35–55(–60) × 7–9 µm. *Ascospores* allantoid, subhyaline to light yellow, 8–10(–11) × 2–2.5 µm. *Colonies* light pink, turning grey and forming numerous pycnidia with age. *Conidia* filiform 16–22(–25) × 1.5–2 µm.

Hosts. *Citrus paradisi* (Australia, NSW), *Vitis vinifera* (Australia, NSW; USA, CA), *Ulmus procera* (Australia, SA).

Notes. This fungus differs from all *Eutypella* species recognized by Rappaz (1987) mostly due to its smaller perithecia (commonly <250 µm). This fungus is also distinctive as a result of the light pink coloration of colonies when grown on PDA and PDA-tet.

Specimens examined. AUSTRALIA, NSW, Hunter Valley, on dead branches of *Citrus paradisi*, Dec. 2008,

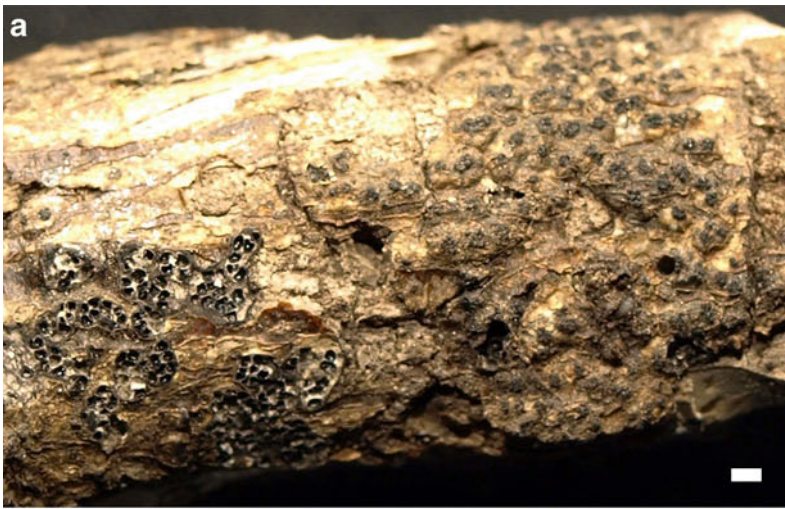
HOLOTYPE: F. P. Trouillas, coll. number HVGRF02, DAR81039, CBS128336; on dead branches of *Vitis vinifera*, Dec. 2008, ISOTYPE: F. P. Trouillas, coll. number HVVIT05, DAR81040, CBS128337.

Discussion

Phylogenetic analyses of both the ITS regions of the rDNA and partial sequence of the β-tubulin gene identified 12 diatrypaceous species from various woody host plants in Australia (shown in bold in Figs. 1 and 2), including the recently described *D. brunneospora* and *E. australiensis* (Trouillas et al. 2010a, b). Comparison with reference sequences obtained from GenBank facilitated the identification of *C. ampelina*, *E. leptoplaca*, and a *Cryptosphaeria* sp. isolated from cankers on *Populus* spp. in NSW and closely related to *Cryptosphaeria lignyota* (Fr.) Auersw. All the remaining species reported from this study were identified based on morphology. *E. leptoplaca* is reported from *Fraxinus angustifolia*, *Schinus molle* var. *areira* and *Populus* spp., although we failed to isolate the pathogen from grapevine despite the existence of previous records from this host in California (Trouillas and Gubler 2004). The occurrence of *E. lata* on naturalized and ornamental plant species in close to vineyards was confirmed. Negligible variations in the sequences of studied genes were found within each species group (or clade), which regularly included isolates both from different host plants and/or continents. ITS and β-tubulin sequences from isolates of *C. ampelina*, *C. rabenhorstii*, *E. lata*, *E. leptoplaca*, *Eutypella citricola* and *E. microtheca* from Australia appeared nearly identical to their California counterparts (Trouillas et al. 2010a, b).

Surveys for diatrypaceous fungi associated with grapevines and other woody hosts in Australia allowed the isolation of original specimens of what appeared to be new species in this family. Hence, *D. vulgaris*, *E. microtheca* and *E. cryptovalsoidea* are described as new species in this paper. Our collections were distinguished from previously described species by their unique morphological characters. *Eutypella microtheca* had exceptionally small perithecia and mycelia on PDA exhibited a pink coloration when grown in culture on PDA. *Diatrypella vulgaris* and *E. cryptovalsoidea* bore unusually long asci, which were also wider than previously recorded; these features differed quite significantly from those described for recognized polysporous species in this family. Isolates WA07CO and WA08CB from grapevine were identified as *C. rabenhorstii* and resemble closely early descriptions of this species by Nitschke (1867) and Saccardo (1882).

This research confirmed the abundance and diversity of Diatrypaceae harbored by grapevines, as shown in a similar



◀ **Fig. 5** Morphology of *Eutypella citricola*. **a.** Pustulate stromata aggregated in the bark of *Citrus sinensis*; **b.** Pustulate stromata on lignified canes of *Vitis vinifera*; **c.** Long-stalked ascus; **d.** Allantoid ascospores; **e.** Colony after one month incubation in the dark at 25°C on 85 mm PDA dish. Bars=1 mm in a; 5 mm in b; 50 µm in c; 20 µm in d

study in California (Trouillas et al. 2010a, b). Among the species reported in the present study, seven were isolated from grapevine wood including *C. ampelina*, *C. rabenhorstii*, *Diatrype* sp., *D. vulgaris*, *E. citricola*, *E. lata* and *E. microtheca*. The incidence and distribution of Diatrypaceae in grapevine cankers varied significantly among the regions surveyed but in many instances these newly reported fungi were more widespread and abundant than *E. lata*. *Eutypa lata* was thought to be the main diatrypaceous species associated with canker diseases in Australia, however, both *E. microtheca* and *E. citricola* appeared to be the more dominant species occurring in grapevine cankers in parts of the Hunter Valley (NSW), where *E. lata* remained elusive. *Eutypella citricola* was found abundantly in both NSW and WA vineyards. In most instances, its presence on grapevines could be explained by the proximity of abandoned citrus orchards and declining citrus trees bearing numerous perithecia of this fungus. Generally, species of Diatrypaceae encountered on grapevines also occurred on other agricultural host plants and ornamentals adjacent to, or in close proximity to vineyards. Furthermore, many of the species commonly found in Australian vineyards were identical to those isolated during previous surveys throughout California vineyards and therefore provided new information on the host range and possible origin of these fungi.

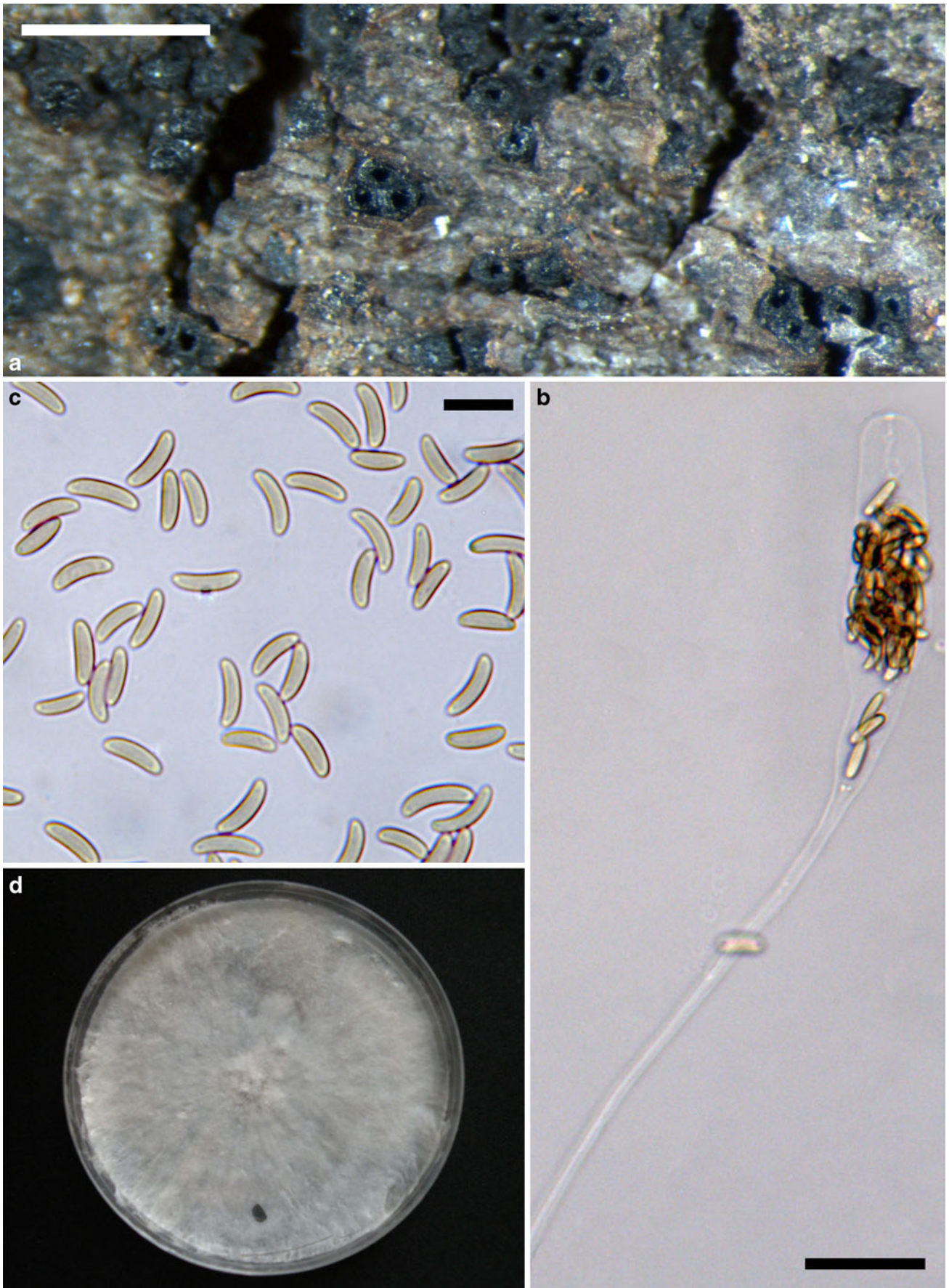
Each genus included in the phylogenetic analyses occurred in more than one clade across the MP trees suggesting polyphyletic origins of diatrypaceous genera. Analyses confirm the observation by Acero et al. (2004) that the current taxonomic scheme for the Diatrypaceae may not reflect the true evolutionary relationships of these fungi and limits between genera. Moreover, the present analyses did not allow the evolutionary history of Diatrypaceae to be elucidated, as bootstrap values were small at deep nodes within the various tree topologies. Increased sampling of taxa (within a monophyletic group) has been widely accepted as a means to increase the average accuracy of phylogenies (Rannala et al. 1998; Pollock et al. 2002; Zwickl and Hillis 2002; Heath et al. 2008). As the diatrypaceous mycota remains poorly investigated worldwide, particularly in tropical regions, exploring the overall diversity of these fungi may be necessary ultimately to resolve the evolutionary relationships in this family. We anticipate that much broader sampling of taxa combined with multigene phylogenies will be necessary in future studies to resolve the evolutionary relationships

within this family. Until then, the assignment of newly discovered species into specific diatrypaceous genera may be provisional.

Number of spores per ascus (eight spores versus more than eight spores) has been used traditionally to delineate genera of the Diatrypaceae. Species with polysporous asci have been assigned to genera including *Diatrypella* and *Cryptovalsa*, which differed from one another mostly by the degree of stromatic tissue produced around the perithecia. Unfortunately, Rappaz did not consider polysporous Diatrypaceae in his work and no modern taxonomic treatment of polysporous Diatrypaceae is available. Moreover, many types for these genera remain out of reach while original descriptions are often inadequate to delineate and identify species. Delineating *Diatrypella* and *Cryptovalsa*, has proved challenging and species are often transferred between the two genera. Wehmeyer (1926) regarded polysporous Diatrypaceae as a distinct phylogenetic lineage. Glawe and Rogers (1984) argued that multisporous species might have evolved independently and repeatedly within this family while Tiffany and Gilman (1965) placed the two names in synonymy. *Diatrypella* has also been considered as a polysporous counterpart of *Diatrype*, and *Cryptovalsa* as a polysporous counterpart of *Eutypa* (Vasilyeva and Stephenson 2005). As demonstrated by the present DNA-based phylogenies, the morphospecies *Cryptovalsa* and *Eutypella* as well as *Diatrype* and *Diatrypella* showed molecular affinities. These results suggest a lack of evolutionary significance of the polysporous ascus feature in the Diatrypaceae.

In this study diatrypaceous strains were commonly isolated from necrotic grapevine wood. Furthermore, certain species normally occurring as saprophytes on the native vegetation in California could occasionally infect wounded active grapevine wood (Trouillas et al. 2010a, b). Fungi in this family are likely to play important ecological functions and may ultimately contribute to the decay of their host plant, thereby affecting plant health and crop longevity. Biologically characterization of these fungi, in term of trophism and ecology, is required to elucidate their role in the decline of the host, particularly in pathosystems involving grapevines and the occurrence of trunk disease complexes. It is important that the pathogenicity of these species on grapevine is determined, and if necessary, management strategies for trunk diseases refined to include these species.

► **Fig. 6** Morphology of *Eutypella cryptovalsoidea*. **a.** Perforated perithecial ostioles emerging singly or in groups through bark of *Ficus carica*; **b.** Long-stalked ascus; **c.** Ascospores; **d.** Colony after one month incubation in the dark at 25°C on 85 mm PDA dish. Bars=1 cm in a; 20 µm in b; 10 µm in c



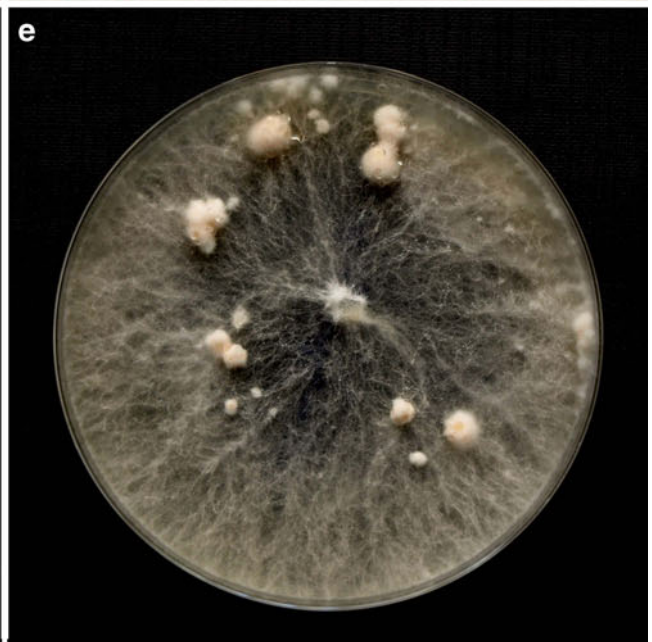
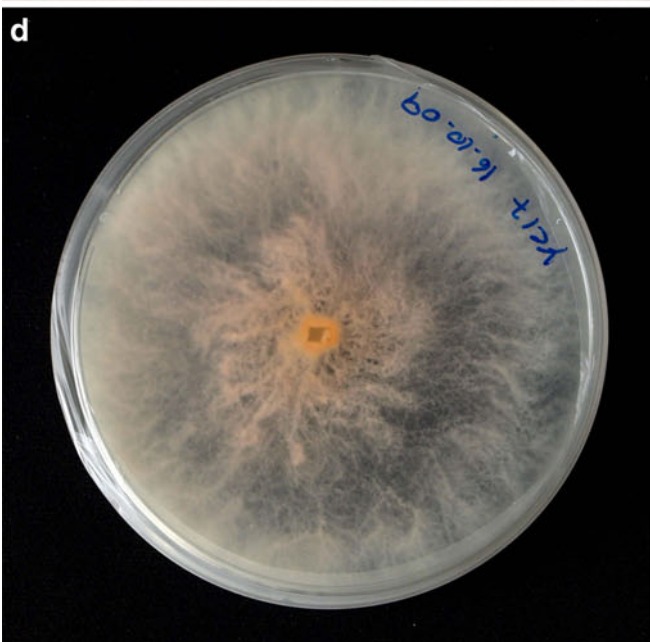


Fig. 7 Morphology of *Eutypella microtheca*. **a.** Stromata in bark of *Citrus paradisi* elevating the periderm surface and minute perithecial cavities; **b.** Long-stalked ascus; **c.** Allantoid ascospores; **d.** Pink underside of colony after 5 days on 85 mm diam PDA dish incubated under intermittent fluorescent lighting (12 h); **e.** Light pink colony with cottony mycelium aggregates after one month incubation in the dark at 25°C on 85 mm PDA dish. Bars=1 mm in a; 50 µm in b; 50 µm in c

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