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Sardiniella urbana gen. *et* sp. nov., a new member of the *Botryosphaeriaceae* isolated from declining *Celtis australis* trees in Sardinian streetscapes

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Abstract

During a survey carried out in 2013 throughout the streets of Sassari (Sardinia, Italy) aimed at clarifying the causes of a decline affecting European hackberry, a collection of a Botryosphaeriaceous taxon was isolated from 14 trees showing sunken cankers with wedge-shaped necrotic sectors and a progressive dieback of shoots and branches as well as collar rot and stem exudates. Although morphologically similar to *Diplodia* and *Dothiorella*, these strains differed from all known species of *Botryosphaeriaceae* in their colony appearance and conidial shapes. A phylogenetic analysis based on combined LSU, ITS and *tef1-α* sequence data showed that these strains form a distinct lineage within the *Botryosphaeriaceae*. Based on molecular phylogeny and morphology, a new genus named *Sardiniella* is herein introduced to accommodate the new *taxon Sardiniella urbana*. Pathogenicity was verified by wound inoculation of 3 year-old seedlings of European hackberry using four different strains. All strains caused sunken cankers and necrotic lesions on inner bark and sap wood tissues in the stems of inoculated seedlings and in all cases the fungus was successfully re-isolated, fulfilling Koch's postulates. Results from the pathogenicity test suggest that this new species is directly involved in the aetiology of the observed decline in European hackberry trees as well as representing a potential risk to public safety in urban environments.

Key words – urban forestry – emerging pathogen – morphology – phylogeny – taxonomy

Introduction

Botryosphaeriaceae species are widely recognized as important fungal plant pathogens worldwide. In particular, several species within this family represent a growing threat to agricultural and forest ecosystems in Mediterranean climate (Gramaje et al. 2012, Lynch et al. 2013, Linaldeddu et al. 2016). The family currently comprises 22 phylogenetically well-defined genera and over one hundred species known from culture and for which molecular data are available in public database (Crous et al. 2013, Phillips et al. 2013, Hyde et al. 2014, Crous et al. 2015). Over the last decade, *Botryosphaeriaceae* has undergone important revision and several new

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species and genera have been introduced chiefly on the basis of combined morphological and multiple gene sequence data (Crous et al. 2006, Phillips et al. 2008, Rojas et al. 2008, Liu et al. 2012, Slippers et al. 2013, Hyde et al. 2014, Crous et al. 2015).

Most of the studies on species of *Botryosphaeriaceae* have focused on woody hosts of economic importance such as eucalypt, grapevine, mango and pine (De Wet et al. 2002, Taylor et al. 2009, Marques et al. 2013, Linaldeddu et al. 2015), while very few studies have investigated species occurrence and distribution as well as damage caused by these fungi on ornamental trees in urban environments (Begoude et al. 2010, Mayorquin et al. 2012). Recently, Zlatković et al. (2016) reported ten *Botryosphaeriaceae* species associated with extensive dieback and mortality of various ornamental trees and shrubs in a small geographical area in the western Balkans. They emphasized that diseases caused by *Botryosphaeriaceae* could be linked to adverse abiotic factors as well as other stresses typical of urban environments, such as air pollution and pruning activity.

In 2013, during a survey carried out throughout the main streets of Sassari (Sardinia, Italy) aimed at clarifying the causes of a decline affecting European hackberry (*Celtis australis* L.), a collection of botryosphaeriaceous strains was isolated from trees showing V-shaped cankers and a progressive dieback of shoots and branches as well as collar rot and stem exudates. Although morphologically similar to *Diplodia* and *Dothiorella*, these strains differed in their colony appearance, conidial shape and DNA sequence data (ITS and *tef1-α*) from all known species of *Botryosphaeriaceae*.

Therefore, the aim of this study was to clarify the morphology, phylogeny, taxonomy and pathogenicity of these fungal isolates collected from European hackberry. This was achieved using a polyphasic approach based on the combination of molecular sequence data of the large subunit (LSU) rDNA gene, ITS and *tef1-α* regions and anamorph morphology. Furthermore, the pathogenicity of this new *Botryosphaeriaceae* member was tested on 3-year-old seedlings of European hackberry.

Materials & Methods

Isolates and morphology

Isolates used in this study were obtained from 14 declining European hackberry trees planted along 3 streetscapes in the city of Sassari (Sardinia, Italy). A total of 22 symptomatic samples were collected. Samples were taken to the laboratory and the outer bark surface tissue was cut away with a scalpel. Longitudinal and transversal cuts from symptomatic samples revealed internal symptoms. Isolations were made from chips of inner bark and xylem tissue (~5 mm^2) removed from the margin of necrotic lesions with a sterile scalpel. All chips were cultured on potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, UK) in Petri-dishes. After incubation at 25°C for 5–7 days in the dark, fungal colonies were sub-cultured onto half-strength PDA supplemented with autoclaved holm oak twigs and incubated at room temperature with natural daylight until pycnidia developed.

Colony growth characteristics including surface and reverse colony appearance were recorded after 7 days of incubation at 25 °C in the dark on PDA, malt extract agar (MEA, 20 g/L malt extract, 20 g/L agar, Oxoid Ltd.), and oatmeal agar (OA, 72.5 g/L, Sigma-Aldrich). Cardinal temperatures for growth were determined on plates of PDA incubated at 5, 10, 15, 20, 25, 30, 35 and 40 °C (±0.5 °C) in the dark. Five replicate plates of four isolates were made and colony diameters were measured after 4 days. For microscopy, the contents of conidiomata were dissected and mounted in 100 % lactic acid. Measurements of conidiogenous cells and conidia were made with the Leica IM 500 measurement module from images recorded with the ×100 objective on a Leica DFC 320 digital camera. Spore dimensions are presented as mean values of 50 conidia with extreme values in parentheses. Dimensions of other structures are given as means of at least 20 measurements.

Representative isolates were stored on PDA slants under oil in the culture collection of the Sez. di Patologia vegetale ed Entomologia, Dipartimento di Agraria at the University of Sassari. A representative culture was deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht,

the Netherlands and nomenclatural data in MycoBank (www.MycoBank.org; Crous et al. 2004) and Faces of Fungi (www.facesoffungi.org; Jayasiri et al. 2015) databases. The holotype was lodged with the herbarium of Instituto Nacional de Investigação Agrária e Veterinária I.P., Oeiras, Portugal (LISE).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 5-day-old cultures grown on PDA at 25°C using Instagene Matrix (BioRad Laboratories, Hercules, California, USA). The entire internal transcribed spacer (ITS) region of the ribosomal DNA, including the 5.8S rRNA gene, was amplified and sequenced using primers ITS1 and ITS4 (White et al. 1990), a partial sequence of the 28S rDNA gene (LSU) region was amplified and sequenced using primers NL1 and NL4 (O'Donnell 1993), whereas part of the *tef1-α* gene encoding translation elongation factor 1 alpha was amplified and sequenced with primers EF446f and EF1035r (Inderbitzin et al. 2010). PCR amplification was carried out as described by Linaldeddu et al. (2013) and the products were purified using the EUROGOLD gel extraction kit according to the manufacturer's instructions (EuroClone S.p.A., Pero, Italy). Both strands were sequenced by BMR Genomics DNA sequencing service (www.bmr-genomics.it). Sequences were edited with FinchTV v1.4.0 (Geospiza, Inc., Seattle, Washington, USA; http://www.geospiza.com/finchtv) and compared with sequences deposited in GenBank using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov). New sequences were deposited in GenBank (Table 1). Alignments and trees are in TreeBase with study ID S19467.

Phylogenetic analyses

The ITS, LSU and *tef1-α* sequences of the isolates obtained in this study were combined and aligned with sequences of 42 *taxa* retrieved from GenBank, representing 18 genera of the family *Botryosphaeriaceae*. Sequence alignments were performed with ClustalX v. 1.83 (Thompson et al. 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). Alignments were checked and manual adjustments made if necessary using BioEdit v. 7.2.5 (Hall 1999). Maximum likelihood (ML) analyses were done using MEGA6 (Tamura et al. 2013) using the best fitting DNA evolution model determined by the program. ML analyses were performed on a Neighbour-Joining starting tree automatically generated by the software. Nearest-Neighbour-Interchange (NNI) was used as the heuristic method for tree inference and 1000 bootstrap replicates were performed. The robustness of the trees was evaluated by 1000 bootstrap replications. *Saccharata proteae* was used as outgroup to root the tree and that was visualised with TreeView v. 1.6.6 (Page 1996).

Table 1 Isolates included in the phylogenetic analyses. Type species of each genus are given in bold typeface and newly generated sequences are indicated in italics.

Species	**Strain**	**GenBank accession number**		
		LSU	**ITS**	*tef1-α*
Barriopsis fusca	CBS 174.26 ex-type	DQ377857	EU673330	EU673296
Botryobambusa fusicoccum	MFLUCC 11-0143 ex-type	JX646809	JX646792	JX646857
	MFLUCC 11-0657	JX646810	JX646793	JX646858
Botryosphaeria agaves	MFLUCC 11-0125 ex-neotype	JX646808	JX646791	JX646856
Botryosphaeria corticis	CBS 119047 ex-epitype	EU673244	DQ299245	EU017539
Botryosphaeria dothidea	CBS 115476 ex-type	AY928047	AY236949	AY236898
Cophinforma atrovirens	MFLUCC 11-0425 ex-type	JX646817	JX646800	JX646865
	CBS 117444	DQ377855	KF531822	KF531801
Diplodia cupressi	CBS 168.87 ex-type	EU673263	DQ458893	DQ458878
Diplodia mutila	CBS 112553	AY928049	AY259093	AY573219
Diplodia rosulata	CBS 116470 ex-type	DQ377896	EU430265	EU430267
Diplodia sapinea	CBS 393.84 ex-epitype	DQ377893	DQ458895	DQ458880

Species	Strain	GenBank accession number		
		LSU	ITS	*tef1-α*
Diplodia seriata	CBS 112555 ex-epitype	AY928050	AY259094	AY573220
Diplodia tsugae	CBS 418.64 ex-isotype	DQ377867	DQ458888	DQ458873
Dothiorella iberica	CBS 115041 ex-type	AY928053	AY573202	AY573222
Dothiorella prunicola	CBS 124723 ex-type	EU673232	EU673313	EU673280
Dothiorella sarmentorum	IMI 63581b ex-type	AY928052	AY573212	AY573235
Endomelanconiopsis endophytica	CBS 120397 ex-type	EU683629	EU683656	EU683637
	CBS 122550	EU683634	EU683664	EU683645
Endomelanconiopsis microspora	CBS 353.97 ex-type	EU683628	EU683655	EU683636
Eutiarosporella tritici	CBS 118719 ex-type	DQ377941	KF531830	KF531809
Lasiodiplodia crassispora	CBS 118741 ex-type	DQ377901	DQ103550	EU673303
Lasiodiplodia gonubiensis	CBS 115812 ex-type	DQ377902	AY639595	DQ103566
Lasiodiplodia rubropurpurea	CBS 118740 ex-type	DQ377903	DQ103553	DQ103553
Lasiodiplodia theobromae	CBS 164.96 ex-neotype	EU673253	AY640255	AY640258
Lasiodiplodia venezuelensis	CBS 118739 ex-type	DQ377904	DQ103547	EU673305
Macrophomina phaseolina	CBS 162.25	DQ377905	KF531826	KF531803
	CBS 227.33	DQ377906	KF531825	KF531804
Marasasiomyces karoo	CBS 118718 ex-type	DQ377939	KF531828	KF531807
Melanops tulasnei	CBS 116805	FJ824764	FJ824769	FJ824774
	CBS 116806	FJ824765	FJ824770	FJ824775
Neodeightonia phoenicum	CBS 122528 ex-type	EU673261	EU673340	EU673309
Neodeightonia subglobosa	CBS 448.91 ex-type	DQ377866	EU673337	EU673306
Neofusicoccum arbuti	CBS 116131 ex-type	DQ377915	AY819720	KF531792
Neofusicoccum luteum	CBS 110299 ex-type	AY928043	AY259091	AY573217
Neofusicoccum mangiferae	CBS 118531	DQ377920	AY615185	DQ093221
Neofusicoccum parvum	CMW 9081 ex-type	AY928045	AY236943	AY236888
Neoscytalidium hyalinum	CBS 145.78 ex-isotype	DQ377922	KF531816	KF531795
	CBS 251.49	DQ377923	KF531819	KF531797
Phaeobotryon mamane	CPC 12440 ex-type	EU673248	EU673332	EU673298
	CPC 12264	DQ377898	EU673331	EU673297
Pseudofusicoccum stromaticum	CBS 117448 ex-type	DQ377931	AY693974	AY693975
	CBS 117449	DQ377932	DQ377932	DQ436936
Saccharata proteae	CBS 115206	DQ377882	KF531812	KF531789
Sardiniella urbana	BL179 = CBS 141580 ex-type	*KX379676*	*KX379674*	*KX379675*
	BL180	*KX379679*	*KX379677*	*KX379678*
	BL181	*KX379682*	*KX379680*	*KX379681*
	BL182	*KX379685*	*KX379683*	*KX379684*
Spencermartinsia citricola	ICMP 16828 ex-type	EU673323	EU673242	EU673290
Spencermartinsia mangiferae	CBS 500.72	EU673237	EU673318	EU673285
Spencermartinsia plurivora	CBS 117006	EU673236	AY905555	AY905562
Spencermartinsia viticola	CBS 117009 ex-type	AY905554	DQ377873	AY905559
Sphaeropsis citrigena	ICMP 16812 ex-type	EU673246	EU673328	EU673294
Sphaeropsis eucalypticola	MFLUCC 11-0579 ex-type	JX646819	JX646802	JX646867
Sphaeropsis porosa	CBS 110496 ex-type	DQ377894	AY343379	AY343340
Sphaeropsis visci	CBS 186.97	DQ377868	EU673325	EU673293

Acronyms of culture collections: CBS: Centraalbureau voor Schimmelcultures, The Netherlands; CMW: M.J. Wingfield, FABI, University of Pretoria, South Africa; CPC: Collection of Pedro Crous housed at CBS; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, New Zealand; IMI: CABI Bioscience, Egham, UK; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; BL: B.T. Linaldeddu culture collection housed at Dipartimento di Agraria, Università di Sassari, Italy.

Fig. 1 – Main symptoms on European hackberry trees growing on a street verge. Progressive dieback of twigs and branches (a); branch with bark necrosis visible at level of a sunken cankers (b); cross section of a branch showing a characteristic wedge-shaped necrotic sector (c); an active sunken canker in the trunk wet with blackish exudates (d); cross section showing extensive necrotic lesion of xylem tissues corresponding to a canker (e).

Pathogenicity test

The pathogenicity of the four isolates investigated in this study, was tested on 3-year-old European hackberry seedlings grown in plastic pots (10 cm diameter, 1.5 l volume). Ten seedlings were inoculated with each isolate, and ten seedlings were used as control. The inoculated region of the stem was surface-disinfected with 70% ethanol and a small piece of outer and inner bark was removed with a flamed scalpel and replaced with an agar-mycelium plug taken from the margin of an actively growing colony on PDA. The inoculation site was covered with cotton wool soaked in sterile water and wrapped in a piece of aluminum foil secured with masking tape. Controls were inoculated with a sterile PDA plug applied as described above. Inoculated seedlings were kept in the laboratory at 18–26°C in natural daylight for 40 days. At the end of experimental period, the outer bark was carefully removed with a scalpel and the length of necrotic lesion surrounding each inoculation site was measured. Re-isolation of the inoculated strains was attempted by transferring onto PDA 10 pieces of inner bark and wood taken from around the margin of each lesion. Cultures were grown in daylight and room temperature until fungal colonies developed.

Statistical analyses

Pathogenicity assay data were checked for normality and then subjected to analysis of variance (ANOVA). Significant differences among mean values were determined using Fisher's least significant differences multiple range test (P = 0.05) after one-way ANOVA using *XL*STAT software (Addinsoft, Paris, France).

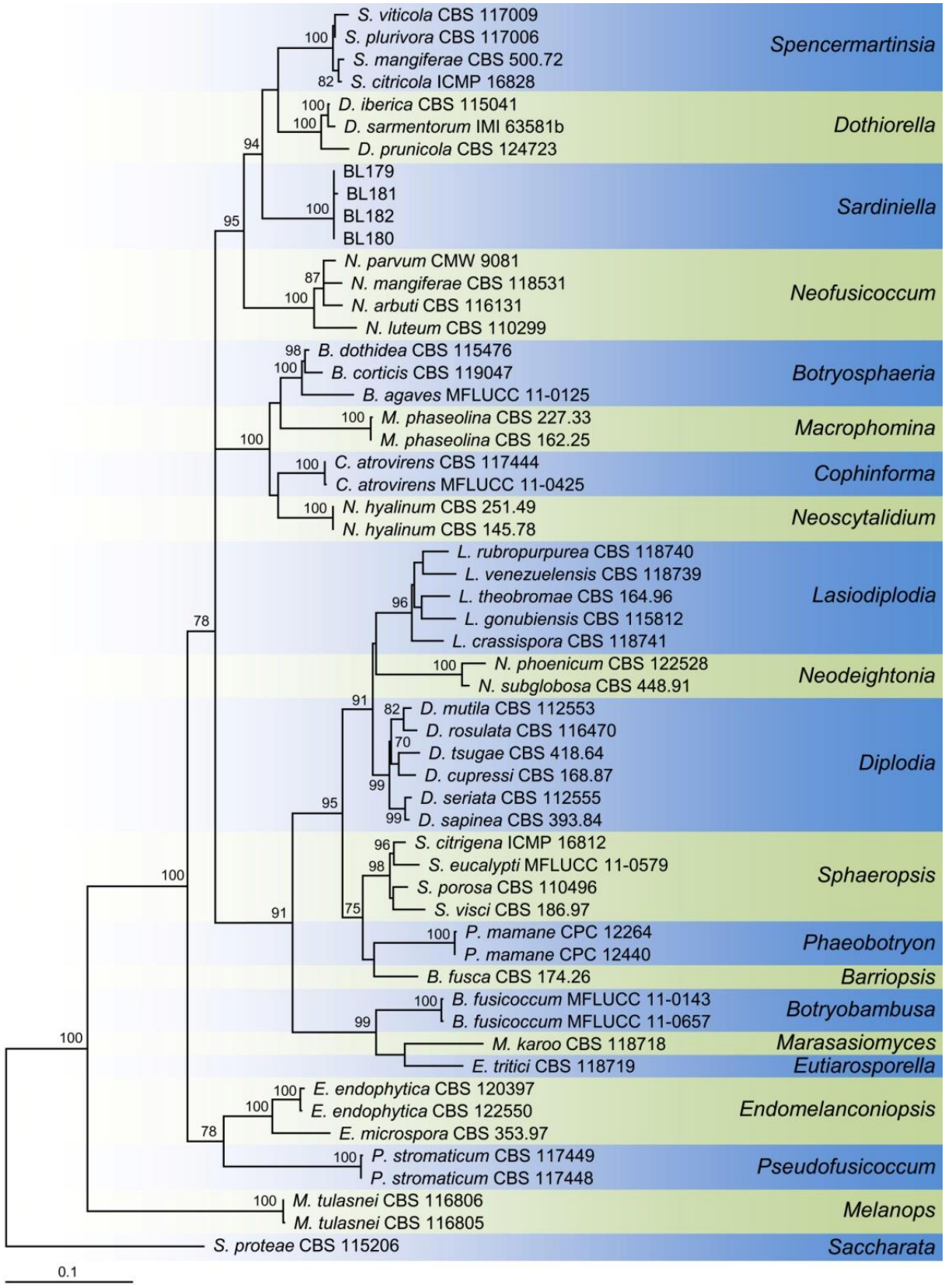

Fig. 2 – Maximum Likelihood tree based on the General Time Reversible model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1000 replicates) are given at the nodes.

Results

Symptoms and isolations

Phytosanitary inspections carried out in three streets in Sassari on 47 European hackberry trees over a two year period revealed the presence of severe disease symptoms typical of *Botryosphaeriaceae* infections in 14 trees (Fig. 1). The main symptoms were a progressive dieback of twigs and branches, bark necrosis and sunken cankers. Wedge-shaped cankers were seen in sections of affected branches. The trunks of two trees, felled for reasons of public safety, were sectioned at several points in order to examine internal symptoms. These revealed V-shaped necrosis of trunks at collar level and brown vascular streaking visible as spots in cross sections of trunks cut at breast height. Isolation carried out from 22 symptomatic samples yielded a total of 22 morphologically identical colonies belonging to the *Botryosphaeriaceae* family. Four isolates obtained from four different trees were used as representatives for further molecular studies. No other fungal species were isolated.

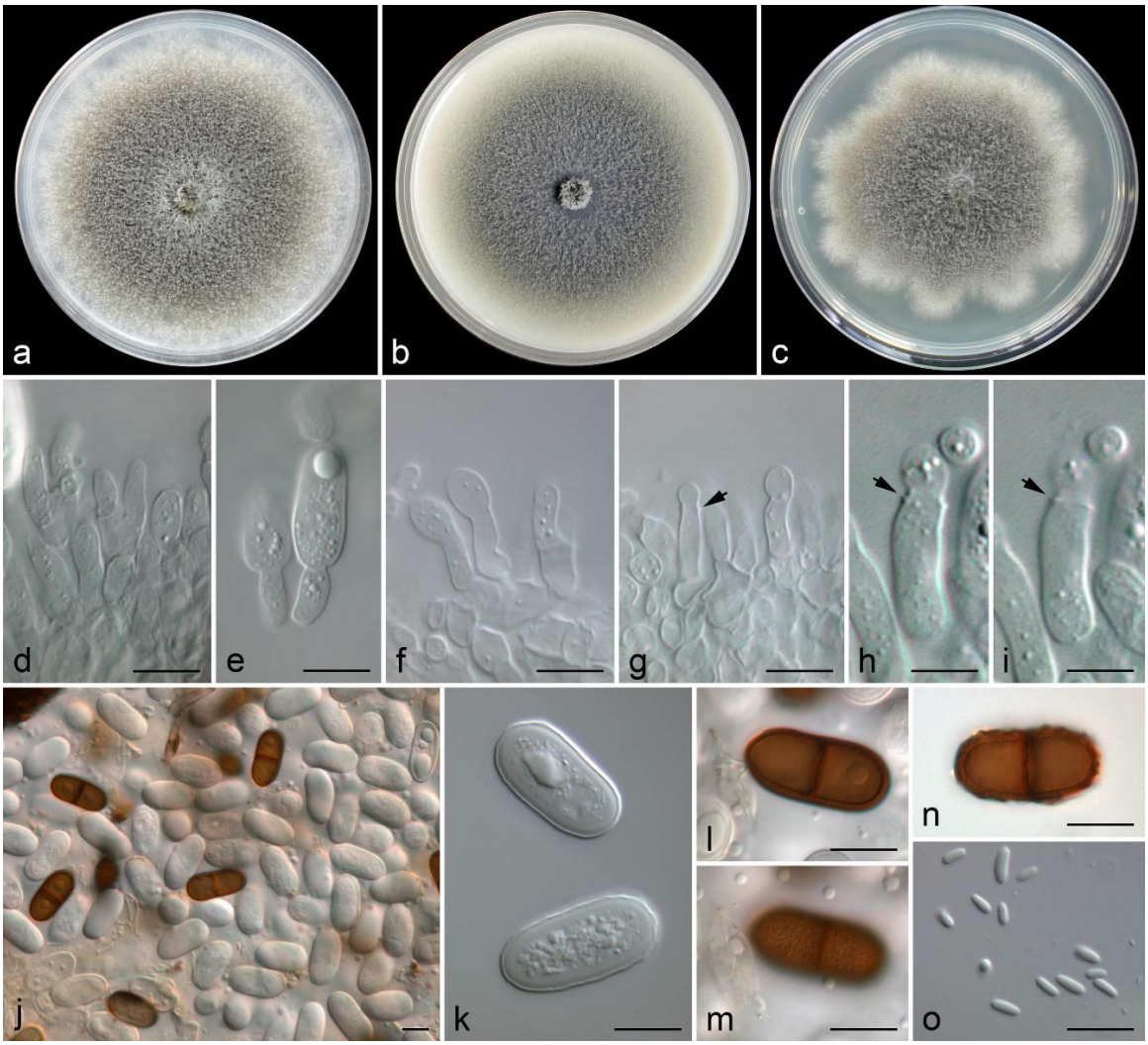

Fig. 3 – Colony morphology of *Sardiniella urbana* after 7 days growth at 25 °C on, a. MEA, b. OA and c. PDA. d. Conidiogenous cells developing on branched conidiophores. e. Conidiogenous cells with developing conidia. f. Young conidiogenous cells. g. Percurrently proliferating conidiogenous cell with a single annellide (arrow). h, i. Percurrently proliferating conidiogenous in two different planes of focus to show a single annellide (arrows). j. Hyaline, aseptate and dark-walled, 1-septate conidia. k. Hyaline, aseptate conidia with undulate wall. l, m. Dark-walled, 1-septate conidium in two different planes of focus to show verruculose inner surface of the wall. n. Verrucose mature conidium. o. Microconidia. Scale bars d–g, j–o = 10 µm; h, i =5 µm.

Phylogenetic analyses

The combined LSU, ITS and *tef1-α* dataset alignment contained 1547 characters. Trees and alignments are available at TreeBase S19467. The ML phylogenetic analysis resolved 19 clades with high bootstrap support (96 to 100%) within the *Botryosphaeriaceae* corresponding to 18 known genera. The isolates from declining European hackberry trees formed a separate clade with high bootstrap support (100%) that clustered between *Neofusicoccum* and *Dothiorella*.

A comparison of *tef1-α* sequences revealed polymorphisms among isolates from European hackberry. In particular, the sequence of the isolate BL181 differed by 3 bp from the sequences of the other strains studied. ITS sequences for all isolates were identical.

Taxonomy

Sardiniella Linaldeddu, A. Alves & A.J.L. Phillips, **gen. nov.**
MycoBank: MB 817511
Facesoffungi number: FoF 02405

Etymology – Named after the island of Sardinia where it was collected.

Type species – ***Sardiniella urbana*** Linaldeddu, A. Alves & A.J.L. Phillips, **sp. nov.**

Parasitic on bark and xylem tissues. *Sexual morph* – not seen. *Asexual morph – Conidiomata* pycnidial, ostiolate, solitary, black, globose, uniloculate, thick-walled, wall composed of brown walled textura angularis. *Conidiophores* hyaline, smooth, thin-walled, cylindrical to oblong, branched. *Conidiogenous cells* hyaline, smooth, cylindrical, holoblastic, proliferating percurrently to form annellides. *Conidia* ovoid to ellipsoid with obtuse ends, thick-walled, sometimes with an irregular/undulate surface, initially hyaline and aseptate, becoming brown and one septate or rarely two septate with age.

Notes – Phylogenetically *Sardiniella* is closely related to *Neofusicoccum* and *Dothiorella/Spencermartinsia* (Fig. 2). The thick-walled, oblong conidia that become pigmented and 1-septate differentiate *Sardiniella* from *Neofusicoccum*. Morphologically, *Sardiniella* is similar to *Diplodia* and *Dothiorella/Spencermartinsia*. However, in *Dothiorella, Spencermartinsia* and in some species of *Diplodia* the conidia become pigmented while still attached to the conidiogenous cell and this character was not seen in *Sardiniella*. In *Diplodia,* the conidial wall is thicker than in *Sardiniella* and these two genera are clearly separated phylogenetically.

Sardiniella urbana Linaldeddu, A. Alves & A.J.L. Phillips, **sp. nov.** Fig. 3

MycoBank: MB 817512
Facesoffungi number: FoF 02406

Etymology – the epithet refers to the urban environments, where the species was originally found.

Sexual morph – not seen. *Asexual morph – Conidiomata* pycnidial, produced on holm oak twigs on ½ PDA within 2–4 weeks, ostiolate, solitary, black, globose, uniloculate, thick-walled, wall composed of dark brown *textura angularis*, lighter in colour towards the inner layers. *Conidiophores* hyaline, smooth, thin-walled, cylindrical to oblong, branched, 5–11 × 3–8 µm. *Conidiogenous cells* hyaline, smooth, cylindrical, holoblastic, proliferating percurrently to form annellides, 5–8 × 2–3 µm. *Conidia* ovoid to ellipsoid, thick-walled sometimes with irregular surface, initially hyaline and aseptate, becoming dark brown and one septate or rarely two septate with age 18.9–(23.5)–26.7 × 10.4–(12)–14.5 µm, ($\bar{x}$ ± S.D. = 23.5 ± 1.48 × 12 ± 0.89 µm, L/W 1.97 ± 0.15; n = 50). *Microconidiogenous cells* not seen. *Microconidia* 2–5 × 0.5–1.5 µm, bacilliform, hyaline, smooth, thin-walled, aseptate, produced in the same conidiomata as conidia.

Culture characteristics – All three culture media supported growth of the fungus. Colonies on PDA reaching 82 mm diameter after 7 days at 25 °C, mycelium velvety and moderately fluffy with an irregular margin, surface initially white and later turning dark olivaceous from the middle of the colony and dark grey in reverse (Fig. 2). On MEA and OA colonies attained 90 mm diameter

before 7 days, the white mycelium was generally aerial and floccose on MEA, but appressed, especially at the margin on OA.

Cardinal temperatures – Min. < 5 °C, max. > 35 °C, opt. 25 °C. All isolates failed to grow at 40 °C.

Known distribution – Sassari, Sardinia (Italy).

Habitat – On trunks and cankered branches of European hackberry (*Celtis australis*).

Material examined – Italy, Sassari, isolated from a branch canker of *Celtis australis*, 9 September 2013, Benedetto T. Linaldeddu, HOLOTYPE LISE 96308, a dried culture sporulating on *Quercus ilex* twigs, culture ex-holotype CBS 141580 = BL179. Other isolates examined are listed in Table 1.

Notes – The conidial wall of *S. urbana* is frequently undulate or irregular. It is not clear if this is a characteristic of the genus or of this particular species.

Fig. 4 – Symptoms on European hackberry seedlings 40 days after inoculation with *Sardiniella urbana* strain BL179 (a, b); BL180 (c, d); BL181 (e, f); BL182 (g, h). Control seedlings (i, j).

Pathogenicity test

All isolates of *S. urbana* proved to be pathogenic on European hackberry, but different levels of aggressiveness were observed among them. At the end of the experimental period, all seedlings inoculated with fungal mycelial plugs displayed sunken dark brown bark lesions that spread up and down the stem from the inoculation site and also penetrated a few millimeters into the sapwood (Fig. 4). The average lesion size differed significantly between isolates (*d.f.* = 3; $F = 48.78$, $P < 0.001$) e.g. the lesions caused by BL182 (mean ± S.D. = 2.9 ± 0.4 cm) were significantly larger than those caused by BL181 (2.2 ± 0.3 cm), BL179 (2.1 ± 0.3 cm) and BL180 (1.1 ± 0.3 cm). The control seedlings remained asymptomatic with only a trace of inner bark discoloration as a wound response at the inoculation point. The pathogen was successfully re-isolated from symptomatic wood and inner bark tissues from all inoculated seedlings, thus fulfilling Koch's postulates.

Discussion

In this study, *Sardiniella* is introduced as a new genus in the *Botryosphaeriaceae*. The type species, *S. urbana*, was isolated from diseased European hackberry trees in an urban environment on the island of Sardinia. Results of phylogenetic analyses provide robust evidence that *Sardiniella* belongs to a separate genus in the *Botryosphaeriaceae* and together with its close allies form a major clade (Fig. 2). This clade includes four genera *Dothiorella*, *Neofusicoccum*, *Sardiniella* and *Spencermartinsia* that morphologically are readily distinguishable by conidial shape. The thin-walled, obovoid to fusiform conidia of *Neofusicoccum*, clearly separate this genus from *Sardiniella*. The hyaline, thick-walled conidia that become pigmented and 1-septate resemble *Dothiorella* and *Spencermartinsia*, but in *Sardiniella* the conidia do not become coloured while attached to the conidiogenous cell. Colouration of conidia before dehiscence is a feature that distinguishes *Dothiorella* and *Spencermartinsia* from all other genera in the *Botryosphaeriaceae*.

European hackberry is the most popular urban tree in the city of Sassari, with more than 1,450 trees planted along the main streets, in parks and in gardens (Achenza 1995). To date, little information is available about the occurrence, distribution and severity of damage caused by fungal pathogens on European hackberry worldwide. Recently, a foliar disease caused by *Sirosporium celtidis* and a decline caused by *Inonotus rickii* were detected on European hackberry in Italy (Cacciola 2000, Annesi et al. 2003).

In this study, out of 47 trees investigated 14 were found to be severely damaged by *S. urbana* infections. The site factors that may have contributed to the outbreak of *S. urbana* infections still remain unknown as well as its ecology. In recent years, reports of epidemic attacks of *Botryosphaeriaceae* species in different natural ecosystems have gradually increased in Sardinia (Andolfi et al. 2012, Alves et al. 2014, Linaldeddu et al. 2014). These findings emphasize how species in this family represent a growing threat to forest trees in Mediterranean region in both urban and natural environments.

All four isolates of this newly recognized species proved to be pathogenic to European hackberry causing lesions on the bark and brown streaks in the wood congruent with field observations. The variability in aggressiveness as well as the occurrence of polymorphisms in the *tef1-α* sequences among isolates of *S. urbana* detected in this study suggests the need to extend the research about the intraspecific variability of this new member of *Botryosphaeriaceae* to assess the presence of pathotypes and evolutionary lineages. Intraspecific variability has been reported for other *Botryosphaeriaceae* species such as *Diplodia corticola* (Linaldeddu et al. 2013), *Diplodia sapinea* (de Wet et al. 2002) and *Diplodia seriata* (Elena et al. 2015).

The findings of this study emphasize the primary role played by this new species in the etiology of the observed decline in European hackberry trees. They also underline the fact that infection represents a potential risk to public safety in urban environments.

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