**CSIRO** PUBLISHING
*Australasian Plant Pathology*, 2010, **39**, 446–452 www.publish.csiro.au/journals/app

# Morphological and molecular characterisation of *Campylocarpon* and *Cylindrocarpon* spp. associated with black foot disease of grapevines in Uruguay

*E. Abreo*[A,B], *S. Martinez*[A], *L. Bettucci*[A] *and S. Lupo*[A]

[A]Laboratorio de Micología, Facultad de Ciencias, Facultad de Ingeniería, Julio Herrera y Reissig 565, Montevideo, Uruguay.
[B]Corresponding author. Email: eabreo@fing.edu.uy

**Abstract.** Black foot disease of grapevines is a problem in most grape-producing regions of the world. This disease affects both young and mature plants, which show retarded growth and eventually die. Species of *Cylindrocarpon* have been identified as the causal agent of this disease. Fungal isolates were obtained from roots of symptomatic plants and plantlets from commercial vineyards in Uruguay. Morphological characteristics on malt extract agar were recorded, and DNA purified. Internal transcribed spacer (ITS) rDNA was amplified with primers ITS 4 and ITS 5 and the amplified fragments were sequenced. Phylogenetic analyses were done with sequences from Uruguay and sequences from GenBank using PAUP* (Phylogenetic Analysis Using Parsimony). Six phylogenetic groups were found that matched six previously known taxa. These groups were supported by morphological characteristics. Isolates were assignable to *Campylocarpon* and *Cylindrocarpon* species. *Ca. pseudofasciculare*, *C. destructans* var. *crassum*, *C. liriodendri*, *C. macrodydimum*, *C. olidum* var. *crassum* and *C. pauciseptatum* represent new records for Uruguay. This work is the first step towards a better understanding and management of black foot disease in local conditions.

**Additional keywords:** *Neonectria*, rootstock, *Vitis*.

## Introduction

Black foot disease of grapevines is a serious disease in most wine- and grape-producing regions of the world. This disease affects both young plants in nurseries and mature plants in vineyards (Halleen *et al*. 2006*a*). The main symptoms of this disease observed in nursery plants are black discolouration and gum inclusions in xylem vessels of affected rootstocks and black streaking of vascular tissues (Scheck *et al*. 1998). The affected vines show low vigour with small trunks and short internodes, a reduction in total foliage and leaf size, with leaves depicting interveinal chlorosis and necrosis (Scheck *et al*. 1998). In adult plants, early in the growing season, affected vines fail to form shoots after winter dormancy, or achieve poor growth, and they can die by mid summer (Halleen *et al*. 2006*a*).

Several *Cylindrocarpon* Wollenw. species have been associated with black foot disease of grapevines (Halleen *et al*. 2004, 2006*a*). The genus *Cylindrocarpon* (teleomorph: *Neonectria* Wollenw.) contains more than 125 described species (Booth 1966; Schroers *et al*. 2008). Some of these are pathogens of a great number of plants in which they invade the host through wounds in stems and roots, and some are known as common saprobes in soils and on dead plant debris.

*Cylindrocarpon destructans* (Zinssm.) Scholten and *Cylindrocarpon obtusisporum* (Cooke & Harkn.) Wollenw. were the species first associated with black foot disease of grapevines (Maluta and Larignon 1991). *C. destructans* was first associated with grapevines in France in the 1960s (Maluta and Larignon 1991). Since then, it has been reported from diseased grapevines in several countries including Italy (Grasso 1984), Portugal (Rego *et al*. 2000), Argentina (Gatica *et al*. 2001), Germany (Fischer and Kassemeyer 2003), New Zealand and South Africa (Halleen *et al*. 2004), Brazil (Garrido *et al*. 2004) and the USA (Petit and Gubler 2005). *C. obtusisporum* has been found in association with grapevines in Italy (Grasso and Magnano Di San Lio 1975) and more recently in the USA (Scheck *et al*. 1998), although it is strongly believed that these were misidentifications (Halleen *et al.* 2004; Petit and Gubler 2005).

The taxonomy of the causal agents of black foot disease has been reviewed recently by Halleen *et al*. (2004). They included morphological characters and phylogenetic analyses of four *Cylindrocarpon* and *Cylindrocarpon*-like taxa, which were isolated from symptomatic and asymptomatic grapevines from different continents. They recognised three main groups. A group of isolates was described as a new species, namely *Cylindrocarpon macrodidymum* Schroers, Halleen & Crous (*Neonectria macrodidyma* Halleen, Schroers & Crous) including isolates from Australia, Canada, New Zealand and South Africa. In addition, *C. macrodidymum* has also been recently reported from the USA (Petit and Gubler 2005), Chile (Auger *et al*. 2007) and Spain (Alaniz *et al*. 2007). The second group of isolates was defined as a new genus: *Campylocarpon*

 10.1071/AP10021 0815-3191/10/050446

Halleen, Schroers & Crous, which contains to date two new species *Ca. fasciculare* Schroers, Halleen & Crous and *Ca. pseudofasciculare* Halleen, Schroers & Crous described from South Africa. The third group of isolates included the *C. destructans*/*Neonectria radicicola* (Gerlach & L. Nilsson) Mantiri & Samuels species complex, which includes isolates from France, New Zealand and South Africa. This complex had been found to be an aggregate of closely related phylogenetic species based on studies of isolates from angiospermous and coniferous hosts (Seifert *et al*. 2003). Later, Halleen *et al*. (2006*b*) studied isolates morphologically assignable to the *C. destructans*/*Neonectria radicicola* complex and concluded that all the isolates from grapevines should be ascribed to *Neonectria liriodendri* Halleen, Rego & Crous, the teleomorph of *Cylindrocarpon liriodendri* J.D. MacDon. & E.E. Butler, a species previously associated with root rot of tulip poplar (*Liriodendron tulipifera*) in the USA (MacDonald and Butler 1981). *C. liriodendri* was recorded in grapevines from France, Portugal, South Africa (Halleen *et al*. 2006*b*), Australia (Whitelaw-Weckert *et al*. 2007), the USA (Dubrovsky and Fabritius 2007; Petit and Gubler 2007), Spain (Alaniz *et al*. 2007) and Iran (Mohammadi *et al*. 2009).

Recently, another species, *Cylindrocarpon pauciseptatum* Schroers & Crous, was associated with grapevines (Schroers *et al*. 2008). This species was isolated from roots of *Vitis* sp. in New Zealand and Slovenia. However, its pathogenicity to grapevines has not been confirmed.

Little is known about the presence, and host range of *Cylindrocarpon* species in Uruguay. Only *Neonectria galligena* (Bres.) Rossman & Samuels was previously recorded from cankers in branches of apple and pear trees (Koch *et al*. 1981). Grapevines showing external symptoms resembling black foot disease (retarded spring growth, decline and death) were consistently collected in a survey that included the most planted wine regions in southern Uruguay. The main goal of this work was to identify the *Campylocarpon* and *Cylindrocarpon* species associated with black foot of grapevines in Uruguay using morphology and DNA sequence data.

## Materials and methods

### Sampling

Samples were taken from bearing and non-bearing grapevines showing external symptoms of general decline without specific darkening of their xylem. A total of 10 established plants older than 5 years old, and 17 young 1-year-old plants were collected from seven commercial vineyards in the southern wine regions of Uruguay.

### Fungal isolations

Roots of symptomatic plants were debarked, cut in segments of 4 cm and surface disinfected by immersion in ethanol 70% for 1 min, NaOCl 4% for 2 min and washed in sterile distilled water. Fragments of 5 × 2 × 2 mm were obtained from xylem tissue and plated on Petri dishes containing potato dextrose agar (PDA, Oxoid, Hampshire, UK) amended with cloranphenicol (100 mg/L). A total of 100 fragments were analysed from symptomatic adult plant materials.

Plantlets from commercial vineyards were cut in 7 segments of 4 cm, debarked and surface disinfected as indicated above. Ten fragments of 5 × 2 × 2 mm obtained from each segment (70 fragments per plant) were plated in Petri dishes containing PDA. A total of 1190 fragments were plated.

Petri dishes were incubated at 25°C and after 7–10 days the emergent colonies were transferred to slants containing fresh PDA. All strains used in this study are maintained at the fungal culture collection in the Laboratorio de Micología, Facultad de Ciencias/Ingeniería, Montevideo, Uruguay.

### DNA extraction, sequencing and phylogenetic analyses

Cultures for DNA extraction were grown on Petri dishes containing PDA until colony size reached 40 mm. Mycelium was harvested with a sterile scalpel and DNA was extracted and purified following the protocol of Lee and Taylor (1990). Internal transcribed spacer (ITS) rDNA was amplified with primers ITS 4 and ITS 5 (White *et al*. 1990), visualised with UV light in agarose gel (1%), and the amplified segments were purified and sequenced by Macrogen (Seoul, Korea).

Nineteen ITS rDNA sequences obtained from the Uruguayan isolates and 41 sequences obtained from GenBank were included in the analyses (Table 1). The GenBank sequences belonged to species associated with black foot in different regions of the world and those from ex-type cultures were preferred when available (Halleen *et al*. 2004, 2006*b*; Schroers *et al*. 2008), as well as other sequences of related *Neonectria* species (Seifert *et al*. 2003; Petit and Gubler 2005). Three *Fusarium* spp. sequences, two from grapevines in Uruguay and one obtained from GenBank as *Fusarium solani* (Mart.) Sacc. were used as outgroup. The obtained sequences were manually edited using MEGA version 4 (Tamura *et al*. 2007) and aligned by means of ClustalW with sequences from GenBank (Thompson *et al*. 1994). Phylogenetic analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2003) for maximum parsimony analysis. Maximum parsimony analysis was performed by means of the heuristic search option with simple taxa additions and tree bisection and reconnection used as the branch-swapping algorithm. All characters were treated as unordered and of equal weight and gaps were treated as missing data. Support for the nodes of the shortest trees was determined by means of 1000 bootstrap analysis replicates (Hillis and Bull 1993). Tree length (TL), consistency index (CI), retention index (RI), and homoplasy index (HI) were calculated.

### Morphological characterisation

Petri dishes of 9 cm in diameter containing different media including 2% malt extract agar (Oxoid), PDA, and water agar 2% (WA, Oxoid) were inoculated in the centre with a disc of mycelium 6 mm diameter taken from the margin of an actively growing colony. Sporulation of colonies grown on WA was enhanced by the addition of sterilised pine needles placed on the colony surface. The cultures were incubated in darkness or under a 12/12-h dark/light cycle illuminated with fluorescent strip lights and near-UV light (366 nm) at 25°C for 14–28 days. Micromorphological characteristics such as shape and size of macroconidia, phialides, chlamydospores and microconidia,

**Table 1. Species of genera *Cylindrocarpon* and *Campylocarpon* included in the phylogenetic analysis**
Bold entries correspond to Uruguayan isolates. * indicates sequences from ex-type cultures

| Strain | Taxon | Location | Host | GenBank accesion ITS rDNA |
|---|---|---|---|---|
| CBS112613* | *Campylocarpon fasciculare* | South Africa, Riebeeck Kasteel | *Vitus vinifera* cv. Cabernet Sauvignon/ Richter 99 | AY677301 |
| CBS113559 | *Campylocarpon fasciculare* | South Africa, Wellington | *V. vinifera* cv. Sultana/143-B Mgt | AY677303 |
| CBS113560 | *Campylocarpon fasciculare* | South Africa, Wellington | *V. vinifera* cv. Pinotage/ Richter 99 | AY677304 |
| **FI2033** | ***Campylocarpon pseudofasciculare*** | **Uruguay, Colonia** | ***V. vinifera* cv. Chardonnay** | **GU198172** |
| **FI2034** | ***Campylocarpon pseudofasciculare*** | **Uruguay, Colonia** | ***V. vinifera* cv. Chardonnay** | **GU198190** |
| **FI2042** | ***Campylocarpon pseudofasciculare*** | **Uruguay, Canelones** | ***V. vinifera* cv. Cabernet Sauvignon/101–14 Mgt** | **GU198173** |
| **FI2044** | ***Campylocarpon pseudofasciculare*** | **Uruguay, Canelones** | ***V. vinifera* cv. Cabernet Sauvignon/101–14 Mgt** | **GU198191** |
| CBS112592 | *Campylocarpon pseudofasciculare* | South Africa, Wellington | *V. vinifera* cv. Pinotage/ Richter 99 | AY677305 |
| CBS112679* | *Campylocarpon pseudofasciculare* | South Africa, Wellington | *V. vinifera* cv. Sultana/Ramsey | AY677306 |
| CBS503.67 | *Cylindrocarpon cylindroides* | Norway | *Abies alba* | AY677261 |
| CBS605.92 | *Cylindrocarpon destructans* var. *crassum* | Germany, Hamburg | *Tilia petiolaris* root | EF607078 |
| CBS537.92 | *Cylindrocarpon destructans* var. *crassum* | Belgium, Liége | *Aesculus hippocastanum* wood | EF607079 |
| CBS773.83 | *Cylindrocarpon destructans* var. *crassum* | Netherlands | Water, in aquarium with *Anodonta* | AY677276 |
| **FI2048** | ***Cylindrocarpon destructans* var. *crassum*** | **Uruguay, Maldonado** | ***V. vinifera* cv. Sauvignon Blanc/SO4** | **GU198182** |
| **FI2049** | ***Cylindrocarpon destructans* var. *crassum*** | **Uruguay, Maldonado** | ***V. vinifera* cv. Sauvignon Blanc/SO4** | **GU198181** |
| Olrim910 | *Cylindrocarpon didymum* | Lithuania | *Fraxinus excelsior* | AY787694 |
| Olrim92 | *Cylindrocarpon didymum* | Lithuania | *Betula pendula* | AY354237 |
| CBS217.67 | *Cylindrocarpon faginatum* | Canada | *Cryptococcus fagi* nymph on *Fagus grandifolia* | AY677277 |
| CBS328.81 | *Cylindrocarpon ianthothele* var. *majus* | Switzerland | Unknown | AY677279 |
| **FI2041** | ***Cylindrocarpon liriodendri*** | **Uruguay, Canelones** | ***V. vinifera* cv. Benitaka/SO4** | **GU198180** |
| CBS110.81* | *Cylindrocarpon liriodendri* | USA | *Liriodendron tulipifera* | DQ178163 |
| CBS117640 | *Cylindrocarpon liriodendri* | Portugal | *V. vinifera* | DQ178166 |
| CBS117526 | *Cylindrocarpon liriodendri* | Portugal | *V. vinifera* | DQ179164 |
| Olrim158 | *Cylindrocarpon lucidum* | Sweden | *Picea abies* | AY805555 |
| **FI2038** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Canelones** | ***V. vinifera* cv. unknown/SO4** | **GU198174** |
| **FI2039** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Canelones** | ***V. vinifera* cv. unknown/SO4** | **GU198175** |
| **FI2040** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Canelones** | ***V. vinifera* cv. Benitaka /SO4** | **GU198176** |
| **FI2043** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Canelones** | ***V. vinifera* cv. Cabernet Sauvignon/101–14 Mgt** | **GU198177** |
| **FI2045** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Canelones** | ***V. vinifera* cv. unknown/ 101–14 Mgt** | **GU198178** |
| **FI2050** | ***Cylindrocarpon macrodidymum*** | **Uruguay, Maldonado** | ***V. vinifera* cv. Tannat/ 101–14 Mgt** | **GU198179** |
| CBS215.67 | *Cylindrocarpon olidum* | Germany | Rotting rhizome of *Asparagus officinalis* | AY677293 |
| **FI2036** | ***Cylindrocarpon olidum* var. *crassum*** | **Uruguay, Colonia** | ***V. vinifera* cv. Chardonnay** | **GU198184** |
| **FI2037** | ***Cylindrocarpon olidum* var. *crassum*** | **Uruguay, Colonia** | ***V. vinifera* cv. Chardonnay** | **GU198183** |

**Table 1.** (*continued*)

| Strain | Taxon | Location | Host | GenBank accesion ITS rDNA |
|---|---|---|---|---|
| CBS216.67 | *C. olidum* var. *crassum* Gerlach | Germany | *Zygocactus* sp. | AY677294 |
| **FI2032** | ***Cylindrocarpon pauciseptatum*** | **Uruguay, Montevideo** | ***V. vinifera* cv. unknown/ 3309 Couderec** | **GU198185** |
| **FI2046** | ***Cylindrocarpon pauciseptatum*** | **Uruguay, Canelones** | ***V. vinifera* cv. Tannat/ 101–14 Mgt** | **GU198186** |
| **FI2047** | ***Cylindrocarpon pauciseptatum*** | **Uruguay, Canelones** | ***V. vinifera* cv. Tannat/ 101–14 Mgt** | **GU198187** |
| CBS100819 | *Cylindrocarpon pauciseptatum* | New Zealand, Tauranga | *Erica melanthera* | EF607090 |
| CBS120171* | *Cylindrocarpon pauciseptatum* | Slovenia, Krsko | *Vitis* sp. | EF607089 |
| CBS120173 | *Cylindrocarpon pauciseptatum* | Slovenia, Krsko | *Vitis* sp. | EF607088 |
| **FI2055** | ***Fusarium* sp.** | **Uruguay, Colonia** | ***V. vinifera* cv. Syrah** | **GU198188** |
| **FI2056** | ***Fusarium* sp.** | **Uruguay, Colonia** | ***V. vinifera* cv. Syrah** | **GU198189** |
| FKCB030 | *Fusarium solani* | – | – | EU314959 |
| CBS279.48 | *Nectria cinnabarina* | – | – | AF163025 |
| GJS 85–39 | *Neonectria coprosmae* | New Zealand | *Metrosideros* sp. | AY295326 |
| CPC12078 | *Neonectria ditissima* | The Netherlands | *Malus* sp. | DQ178169 |
| CBS112456 | *Neonectria lucida* | USA, Puerto Rico | Bark of recently dead *Cecropia* sp. | AY677296 |
| CBS112615* | *Neonectria macrodidyma* | South Africa, Wellington | *V. vinifera* Sultana/143-B Mgt | AY677290 |
| CBS120170 | *Neonectria macrodidyma* | Slovenia, Nova Gorica | *Vitis* sp. | EF607091 |
| | *Neonectria radicicola* | Costa Rica | *Scolytodes unipunctatus* | AM267272 |
| AR2553 | *Neonectria radicicola* | Venezuela | Bark | AF220968 |
| CTR 71–322 | *Neonectria radicicola* | Venezuela | Unknown | AF220969 |
| CD1287 | *Neonectria radicicola* | – | *Panax* | AY295320 |
| CD1557 | *Neonectria radicicola* | – | *Panax* | AY295329 |
| CBS264.65* | *Neonectria radicicola* | Sweden | *Cyclamen persicum* | AY677273 |
| | *Neonectria ramulariae* Wollenw. | Italy | *Tuber brumale* | DQ350126 |
| TPPH-32 | *Neonectria rugulosa* | Japan, Kanagawa | *Myrica rubra* canker | AB233176 |
| CBS112467 | *Neonectria trachosa* | Scotland | Conifer bark | AY677297 |
| H97519 | *Neonectria veuillotiana* | – | – | EF121866 |
| CBS316.34 | *Neonectria galligena* | Canada | *Betula lutea* | AY677278 |

were recorded. At least 30 conidia were measured for each species.

## Results

A total of 19 isolates assignable to *Cylindrocarpon sensu lato*, including *Campylocarpon* and *Cylindrocarpon* species, were obtained from 27 plants in 7 vineyards.

### *Phylogenetic analyses and morphological characterisation*

The adjusted ITS alignment contained 57 ingroup taxa and three *Fusarium* spp. used as outgroup taxa. Out of the 537 total characters including alignment gaps, 217 were parsimony informative, 76 were variable and parsimony uninformative and 244 were constant. Maximum parsimony analyses of the ITS data yielded 260 equally parsimonious trees where TL = 794 steps, CI = 0.616, RI = 0.867 and HI = 0.384. The 50% majority rule consensus tree obtained is shown in Fig. 1.

Sequences of Uruguayan *Cylindrocarpon* isolates clustered into six well supported groups with sequences obtained from GenBank.

The first group, comprising of four strains, formed a high supported clade (100%) with *Ca. pseudofasciculare* sequences including the ex-type strain. These isolates were characterised by large, curved macroconidia bearing 2–5 septa and measuring 33–53 × 5.5–7.0 µm. Chlamydospores were spherical and microconidia were not observed. The combination of formerly described characters and the presence of chlamydospores indicate that these isolates correspond to *Ca. pseudofasciculare*. The presence of chlamydospores remains the main character for separating the only two known species in the genus.

The second group, comprising of two isolates (FI2036, FI2037), clustered with a sequence of *Cylindrocarpon olidum* var. *crassum* Gerlach, from GenBank (CBS216.67) with a high support value (100%).

The third group of six isolates formed a highly supported subclade (94% bootstrap support) with *N. macrodidyma*/

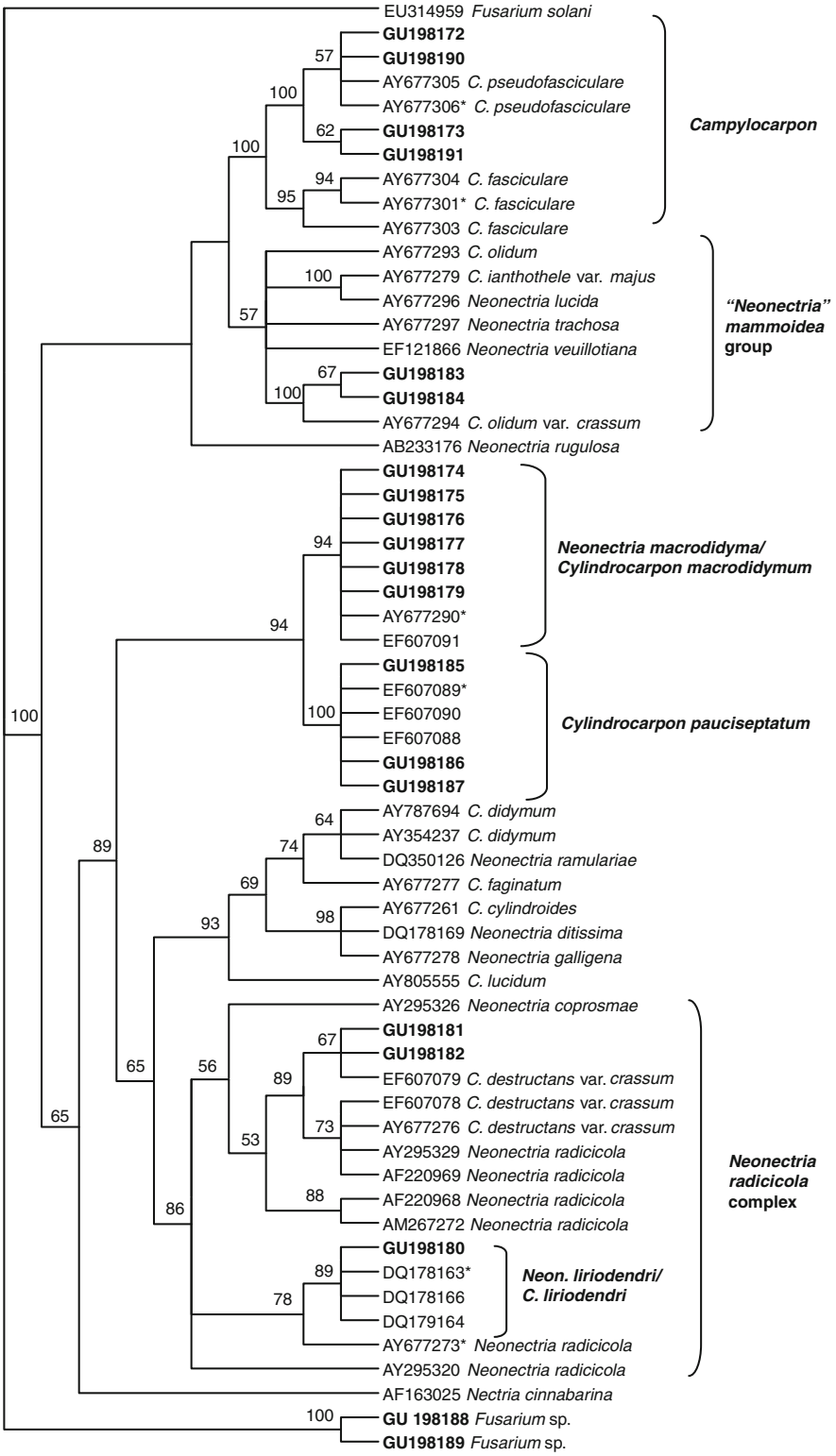


**Fig. 1.** Fifty percent majority rule consensus maximum parsimony phylogenetic tree obtained using internal transcribed spacer sequences of *Campylocarpon* and *Cylindrocarpon* species obtained in the present study and from GenBank. Strain numbers in bold were sequenced for the present analysis. Sequences from ex-type cultures are indicated by *. Bootstrap support values based on 1000 replicates are shown at the nodes.

*C. macrodidymum* sequences obtained from GenBank, including those from the ex-type strain (CBS112615). These isolates were characterised by macroconidia with 1–3 septa, measuring 21.0–40.0 × 5.0–6.5 µm, oval to ellipsoid microconidia with 0–1 septa measuring 10.0–12.0 × 3.5–4.5 µm.

The fourth group of three isolates clustered with *C. pauciseptatum* isolates (100% bootstrap support), including CBS120171, ex-type strain. Macroconidia of these isolates were mostly three septated 38–54 × 6.5–9.0 µm, microconidia 4–6 × 3–4 µm, and chlamydospores 8–12 × 7–12 µm.

The fifth group with two isolates (FI2048, FI2049) clustered in a subclade (89% bootstrap support) containing sequences of the *N. radicicola* complex obtained from hosts other than grapevines, although not the ex-type of this species. The Uruguayan strains clustered with a 67% bootstrap support with one sequence of *Cylindrocarpon destructans* var. *crassum* obtained from GenBank (EF607079). The Uruguayan isolates were characterised by macroconidia with 1–3 septa (macroconidia with 1–2 septa measuring 22–30 × 5.0–6.5 µm and 3 septated macroconidia measuring 34–44 × 6–8 µm), microconidia of 8–13 × 2.5–5.0 µm and brown, spherical chlamydospores 12–16 × 11–13 µm.

The sixth group with Uruguayan isolate FI2041 formed a fourth well supported (89%) subclade with three sequences obtained from GenBank including the ex-type of *Neonectria liriodendri*. This isolate was characterised by macroconidia mostly with 3 septa, occasionally bearing 1 or 2 septa. The 3-septate macroconidia measured 32.0–44.0 × 5.5–6.5 µm. Microconidia and spherical chlamydospores were observed in this isolate. The former measured 6–7 × 3–3.5 µm and the latter measured 8–15 µm in diameter.

The relatively small sample size used in this work was enough to identify 6 species. Among 19 isolates, 6 belonged to *C. macrodidymum* and 3 were obtained from SO4 and 3 from 101–14 Mgt rootstocks; 5 belonged to *Ca. pseudofasciculare*, 2 were obtained from 101–14 Mgt and 3 from an unknown rootstock; 2 *C. destructans* var. *crassum* and 1 *C. liriodendri* were isolated from SO4 rootstock; 3 *C. pauciseptatum* were isolated, 2 from 101–14 Mgt and 1 from 3309C; 2 *C. olidum* var. *crassum* were isolated from an unknown rootstock.

## Discussion

The six different phylogenetic groups found in Uruguay matched with six previously known taxa (Halleen *et al*. 2004, 2006*b*; Schroers *et al*. 2008). The species *Ca. pseudofasciculare*, *C. destructans* var. *crassum*, *C. liriodendri*, *C. macrodydimum*, *C. olidum* var. *crassum* and *C. pauciseptatum* represent new records on grapevine in Uruguay. The identifications were supported by morphological characteristics and ITS rDNA sequence data, except for the two sequences of *C. olidum* var. *crassum* whose original cultures were not available for a morphological study.

Three of the four most pathogenic species associated with black foot were found in Uruguay: *C. liriodendri*, *C. macrodydimum* and *Ca. pseudofasciculare* (Halleen *et al*. 2004, 2006*a*, 2006*b*; Alaniz *et al*. 2009).

*C. liriodendri*, frequently isolated in France, South Africa and Spain (Halleen *et al*. 2006*b*; Alaniz *et al*. 2007, 2009), was found once. However, this could be the result of an underestimation due to the small sample size used in this work.

*C. macrodidymum* is the most frequently isolated species in the USA, Spain and Chile (Petit and Gubler 2005; Alaniz *et al*. 2007; Auger *et al*. 2007). In spite of the small sample size, it appears as it might be also the most frequent in Uruguay, and the only species found in a Uruguayan nursery (Abreo *et al*. 2009). This, together with the fact that this species was also the most prevalent in SO4 rootstock, the most planted one in Uruguay, could help explain its higher frequency in vineyards.

*Ca. pseudofasciculare*, along with the type of the genus *Ca. fasciculare* was recently described as a new species in a new genus, without known teleomorph, from asymptomatic roots of nursery grapevines in South Africa (Halleen *et al*. 2004). Although originally isolated from asymptomatic roots the inoculation of 6-month-old potted grapevine rootstocks with this species showed a reduction of root and shoot mass after 4.5 months (Halleen *et al*. 2004). In Uruguay, *Ca. pseudofasciculare* was isolated only from symptomatic plants.

The sequences of isolates FI 2048 and FI 2049 obtained from symptomatic grapevines and identified as *C. destructans* var. *crassum* clustered with a sequence from GenBank (EF607079) isolated from *Aesculus hippocastanum*. In addition this species was also recorded from other substrates such as *Cocos*, *Lilium*, *Taxus* and *Ulmus* from Europe and Jamaica (Booth 1966). However, strain EF607079 is not the ex-type culture and, therefore, it is not possible to confirm that Uruguayan isolates from grapevines belong to this species. Further studies such as comparison with the ex-type culture sequence are required to confirm its identity. Alternatively, FI 2048 and FI 2049 isolates might represent a species related to the *C. destructans* complex but different from *C. destructans* var. *crassum.* In this regard, Uruguayan isolates show macroconidia with narrower diameter than those originally described for this species (Booth 1966). Even though isolated from symptomatic grapevines in Uruguay, its role as a pathogen still needs to be tested in artificial inoculations.

Finally, *C. pauciseptatum* was recorded only in Slovenia and New Zealand in decayed roots of *Vitis* sp. (Schroers *et al*. 2008). Its presence in Uruguay suggests a larger distribution than previously recorded.

The results from this work contribute towards relevant information regarding the identification of species of *Campylocarpon* and *Cylindrocarpon* associated with black foot symptoms of grapevines in Uruguay. This is the first step towards a better understanding and management of the disease in local conditions.

Further works is required in order to identify, quantify, and determine the distribution of these species in Uruguayan vineyards.

## Acknowledgements

Authors are grateful to INIA-FPTA, ANII Agencia Nacional de Investigación e Innovación, and PEDECIBA, Universidad de la República, Uruguay.

## References


Abreo E, Martínez S, Bettucci L, Lupo S (2009) Especies de Campylocarpon y Cylindrocarpon asociadas al síntoma de pie negro de *Vitis vinifera* en Uruguay. In 'XV Congreso Latinoamericano y XVIII Congreso Chileno de Fitopatología'. Pontifica Universidad Católica de Chile, Santiago de Chile. January 12–16 2009. Abstracts, p. 261. (Asociación Latinoamericana de Fitopatología/Sociedad Chilena de Fitopatología)

Auger J, Esterio M, Pérez I (2007) First report of Black Foot disease of grapevine caused by *Cylindrocarpon macrodidymum* in Chile. *Plant Disease* **91**, 470. doi:10.1094/PDIS-91-4-0470A

Alaniz S, León M, Vicent A, García-Jiménez J, Abad-Campos P, Armengol J (2007) Characterization of *Cylindrocarpon* species associated with Black Foot disease of grapevine in Spain. *Plant Disease* **91**, 1187–1193. doi:10.1094/PDIS-91-9-1187

Alaniz S, Armengol J, León M, García-Jiménez J, Abad-Campos C (2009) Analysis of genetic and virulence diversity of *Cylindrocarpon liriodendri* and *C. macrodidymum* associated with black foot disease of grapevine. *Mycological Research* **113**, 16–23. doi:10.1016/j.mycres.2008.07.002

Booth C (1966) The genus *Cylindrocarpon. Mycological Papers* **104**, 1–56.

Dubrovsky S, Fabritius AL (2007) Occurrence of *Cylindrocarpon* spp. in nursery grapevines in California. *Phytopathologia Mediterranea* **46**, 84–86.

Fischer M, Kassemeyer HH (2003) Fungi associated with Esca disease of grapevine in Germany. *Vitis* **42**, 109–116.

Garrido LR, Sônego OR, Urben AF (2004) *Cylindrocarpon destructans* causador do 'Pie-Preto' da videira no Rio Grande do Sul. *Fitopatologia Brasileira* **29**, 548–550. doi:10.1590/S0100-41582004000500014

Gatica M, Césari C, Magnin S, Dupont J (2001) *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in vines showing 'hoja de malvón' and young vine decline symptoms in Argentina. *Phytopathologia Mediterranea* **40**, S317–S324.

Grasso S (1984) Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. *Informatore Fitopatologico* **1**, 59–63.

Grasso S, Magnano Di San Lio G (1975) Infezioni di *Cylindrocarpon obtusisporium* su piante di vite in Sicilia. *Vitis* **14**, 36–39.

Halleen F, Schroers H-J, Groenewald JZ, Crous PW (2004) Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Studies in Mycology* **50**, 431–455.

Halleen F, Fourie PH, Crous PW (2006*a*) A review of black foot disease of grapevine. *Phytopathologia Mediterranea* **45**, S55–S67.

Halleen F, Schroers H-J, Groenewald JZ, Rego C, Oliveira H, Crous PW (2006*b*) *Neonectria liriodendri* sp. nov., the main causal agent of black foot disease of grapevines. *Studies in Mycology* **55**, 227–234. doi:10.3114/sim.55.1.227

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**, 142–152.

Koch L, Boasso C, Riccio O, Gandolfo C (1981) Enfermedades de las plantas, hongos superiores y saprofitas en el Uruguay. Departamento de Comunicaciones. Dirección de Sanidad Vegetal. M. A. P.

Lee S, Taylor J (1990) Isolation of DNA from fungal mycelia and single spores. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Snindky, TJ White) pp. 282–287. (Academic Press, Inc.: New York)

MacDonald JD, Butler EE (1981) *Cylindrocarpon* root rot of tulip poplar. *Plant Disease* **65**, 154–157.

Maluta DR, Larignon P (1991) Pied-noir: mieux vaut prévenir. *Viticulture* **11**, 71–72.

Mohammadi H, Alaniz S, Banihashemi Z, Armengol J (2009) Characterization of *Cylindrocarpon liriodendri* associated with Black Foot disease of grapevine in Iran. *Journal of Phytopathology* **157**, 642–645. doi:10.1111/j.1439-0434.2009.01555.x

Petit E, Gubler WD (2005) Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevine in California. *Plant Disease* **89**, 1051–1059. doi:10.1094/PD-89-1051

Petit E, Gubler WD (2007) First report of *Cylindrocarpon liriodendri* causing Black Foot disease of grapevine in California. *Plant Disease* **91**, 1060. doi:10.1094/PDIS-91-8-1060A

Rego C, Oliveira H, Carvalho A, Phillips A (2000) Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* **39**, 76–79.

Scheck HJ, Vasquez SJ, Gubler WD (1998) First report of black-foot disease, caused by *Cylindrocarpon obtusisporium*, of grapevine in California. *Plant Disease* **82**, 448. doi:10.1094/PDIS.1998.82.4.448A

Schroers H-J, Zerjav M, Munda A, Halleen F, Crous PW (2008) *Cylindrocarpon pauciseptatum* sp. nov., with notes on *Cylindrocarpon* species with wide, predominantly 3-septate macroconidia. *Mycological Research* **112**, 82–92. doi:10.1016/j.mycres.2007.10.004

Seifert KA, McMullen CR, Yee D, Reeleder RD, Dobinson KF (2003) Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans. Phytopathology* **93**, 1533–1542. doi:10.1094/PHYTO.2003.93.12.1533

Swofford DL (2003) 'PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.' (Sinauer Associates: Sunderland, MA)

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599. doi:10.1093/molbev/msm092

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680. doi:10.1093/nar/22.22.4673

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Snindky, TJ White) pp. 315–322. (Academic Press, Inc.: New York)

Whitelaw-Weckert MA, Nair NG, Lamont R, Alonso M, Priest MJ, Huang R (2007) Root infection of *Vitis vinifera* by *Cylindrocarpon liriodendri* in Australia. *Australasian Plant Pathology* **36**, 403–406. doi:10.1071/AP07041


Manuscript received 28 January 2010, accepted 20 May 2010