Eur J Plant Pathol (2014) 138:347–359
DOI 10.1007/s10658-013-0334-0

Ilyonectria palmarum sp. nov. causing dry basal stem rot of Arecaceae

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Accepted: 28 October 2013 / Published online: 21 November 2013

Abstract During surveys conducted in 2010–2013, a complete breakage or bending of the trunk and a dry basal stem rot were observed on containerised *Brahea armata*, *B. edulis*, *Howea forsteriana* and *Trachycarpus princeps* plants in different nurseries located in eastern Sicily (southern Italy). A cylindrocarpon-like species was consistently obtained from diseased palm tissues, while known pathogens of these hosts such as *Ganoderma, Phytophthora* and *Thielaviopsis* were not found associated with symptomatic tissues or isolated on standard or selective media. A total of 40 cylindrocarpon-like isolates were collected and characterised based on morphology and DNA phylogeny. Multigene analyses based on the β-tubulin, histone H3, translation elongation factor 1-α, and the internal transcribed spacers (ITS1, 5.8S, ITS2) genes facilitated the identification of a new species, described here as *Ilyonectria palmarum*. The pathogenicity of one representative isolate collected from each palm species was tested on plants cultivated under nursery conditions and in a growth chamber. All isolates were pathogenic to *B. armata*, *B. edulis*, *H. forsteriana*, and *T. princeps* and symptoms identical to that observed in nurseries were reproduced. Dry basal stem rot and stem bending caused by *Ilyonectria palmarum* represents a potentially serious problem for nurseries cultivating containerised palms.

Keywords Cylindrocarpon-like asexual morph · Multi-gene analysis · Pathogenicity · Trunk bending · Trunk breakage

Electronic supplementary material The online version of this article (doi:10.1007/s10658-013-0334-0) contains supplementary material, which is available to authorized users.

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Introduction

Basal stem or butt rot is one of the major diseases causing losses in oil and ornamental palms, and has been reported from the USA, Malaysia and South East Asia (Elliott and Broschat 2001; Wong et al. 2012). This disease is caused by several species of *Ganoderma* including *G. zonatum* Murrill, *G. boninense* Pat., and *G. miniatocinctum* Steyaert (Miller et al. 1999; Elliott 2004; Chong et al. 2011; Wong et al. 2012). Ganoderma butt rot caused by *G. zonatum* is the most common and lethal disease of ornamental palms throughout Florida and it has been observed on all palm species in both natural and landscaped settings (Downer et al. 2009). *Ganoderma zonatum* degrades or rots the lower 4–5 ft of the trunk and other symptoms may include wilting or a general decline. Cross sections of infected trunks show a central area that is markedly different in colour (Elliott 2004). Diagnostic basidiomata are not always produced on diseased palms. The disease can therefore often only be confirmed when the palm is removed and the lower trunk sections are examined (Downer et al. 2009). Other lethal palm diseases producing trunk or crown rot are caused by species of *Phytophthora* and *Thielaviopsis* (Uchida 2004; Elliott 2006; Downer et al. 2009). *Phytophthora* species cause several disease symptoms including seedling blight and damping-off, root, stem, collar and bud rots. Phytophthora bud (heart) rot and root rot caused by *P. palmivora* (E.J. Butler) E.J. Butler and *P. nicotianae* Breda de Haan is widespread in Italian nurseries on *Trachycarpus fortunei* (Hooker) H. Wendl. (Cacciola et al. 2011; Polizzi unpublished). Thielaviopsis trunk infections frequently initiate in the upper part of the trunk and transverse sections show symptoms very similar to those induced by *Ganoderma* spp. (Simone 2004; Polizzi et al. 2006). On some species such as coconut and kentia palms a reddish stem bleeding may also occur (Simone 2004; Polizzi et al. 2007).

During 2010, a new basal stem rot was detected on approximately 25 % of 2,000 5-year-old containerised *Brahea armata* S. Watson palms in a commercial nursery located in Praiola, Giarre (Catania province, eastern Sicily, Italy). The symptomatic plants showed a complete breakage or bending of the trunk as a consequence of a partial decay of one side of the basal stem (Fig. 1a–c). The root system appeared healthy, with no significant symptoms of decay (Fig. 1g). Sometimes, a new leaf started from the basal stem below the decayed tissues. Transverse sections through infected trunks showed a brown central area resembling Ganoderma infection, but the decay started from the external part of the trunk (Fig. 1e–f). In addition, no symptoms of general chlorosis, foliage necrosis, and fruiting bodies or conks were detected on infected plants. In relation to this newly discovered disease, the aims of the present study were to: determine the distribution of this disease in Sicilian nurseries; to identify the pathogen(s) associated with the disease via morphological and molecular characterisation; and to verify the pathogenicity of the microorganism(s) associated with the disease.

Materials and methods

Field survey and isolation

During 2010–2013, surveys were conducted on different Arecaceae ornamental palms in 15 nurseries located in eastern Sicily. A total of approximately 300.000 2- to 8-year-old potted plants of *B. armata* (20.000 plants), *B. edulis* H. Wendl. (5.000 plants), *Howea forsteriana* C. Moore & F. v. Muell. (20.000 plants), *T. fortunei* (50.000 plants), *T. princeps* Gibbons, Spanner & San Y. Chen (1.000 plants), *Chamaerops humilis* L. (50.000 plants), *Phoenix canariensis* hort. ex Chabaud (90.000 plants), *P. roebelenii* O' Brien (10.000 plants), *Arecastrum romanzoffianum* (Cham.) Becc. (15.000 plants), and *Washingtonia robusta* (Lindl.) H. Wendl. (40.000 plants) were surveyed for disease symptoms. Plants showing a complete breakage or bending of the trunk were randomly collected for analysis. The disease incidence on each host species from different nurseries was recorded. Infected basal stem tissues collected from symptomatic plants were surface disinfected with 1.5 % sodium hypochlorite for 1 min, rinsed in distilled water, and plated on Ganoderma selective medium (Ariffin and Idris 1991), carrot agar amended with 500 μl of streptomycin sulphate, acidified (lactic acid; pH=3.6) potato dextrose agar (PDA, Oxoid) and PDA amended with 100 μl of streptomycin sulphate and Phytophthora selective medium PARPH (Jeffers and Martin 1986), and incubated at 25 °C.

Morphological characterisation

A total of 40 cylindrocarpon-like isolates were derived from single conidia and maintained on PDA slants (Table 1). For morphological identification all isolates

Fig. 1 Dry basal stem rot caused by *Ilyonectria palmarum* sp. nov. on containerised palms. **a** Trunk bending on *Brahea armata* plants. **b** Trunk bending and death of *B. armata* plant. **c** Breakage of trunk on *Howea forsteriana* plant. **d** Dry basal stem rot with external tissue proliferation on *B. armata* plant. **e**–**f** Discolouration due to degrading tissue remains externalized within the trunk. **g** Root system appears healthy

were grown for 1 week at 25 °C in the dark on Petri dishes containing PDA, and synthetic nutrient-poor agar (SNA) (Crous et al. 2009). Observations were done by mounting fungal structures in clear lactic acid. For each isolate, 30 measurements at × 1,000 magnification were made for each structure using a Zeiss Axio Imager 2 light microscope with differential interference contrast (DIC) illumination, and images captured via an

Table 1 List of 40 cylindrocarpon-like isolates collected from containerised Arecaceae palms showing symptoms of dry basal stem rot

Isolate DiGeSA number[a]	Host	Geographical location
BRA1[b], BRA2, BRA3, BRA4	*Brahea armata*	Giarre, Sicily, Italy, nursery 1
BRA5[b], BRA6, BRA7, BRA8, BRA9	*B. edulis*	Riposto, Sicily, Italy, nursery 1
BRA10, BRA11, BRA12, BRA13, BRA14, BRA15, BRA16, BRA17, BRA18, BRA19, BRA20, BRA21, BRA22	*B. armata*	Giarre, Sicily, Italy, nursery 2
TP1[b], TP2, TP3, TP4,TP5,TP6, TP7, TP8, TP9	*Trachycarpus princeps*	Riposto, Sicily, Italy, nursery 2
HF1, HF2, HF3[b], HF4, HF5, HF6, HF7, HF8, HF9	*Howea forsteriana*	Aci Castello, Sicily, Italy

[a] DiGeSA Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Catania, Italy

[b] Isolates used for the pathogenicity trials

AxioCam MRc5 camera and Zen software. Culture characteristics (texture, density, and zonation) were described on PDA after incubation at 25 °C in the dark for 2 weeks. Colony colours were determined according to Rayner (1970). Cardinal temperatures for growth were determined by inoculating 90-mm diam PDA dishes with a 3-mm diam plug cut from the edge of an actively growing colony. Colony diameters were determined after 1 week in two orthogonal directions. The strains were incubated at 4, 10, 15, 20, 25, 30, 35 °C with three replicate plates per strain at each temperature.

DNA isolation, PCR and phylogeny

Species level identification was obtained by DNA sequencing and phylogenetic analyses of partial β-tubulin (*benA*), histone H3 (*HIS3*) and translation elongation factor 1-α (*TEF-1a*) gene sequences of the 40 strains tested in this study. Each fungal strain was grown in shake culture (150 rpm, 25 °C, 2 days) on Wickerham medium, containing 40 g glucose, 5 g peptone, 3 g yeast extract, 3 g malt extract and up to 1 l water; the mycelium was subsequently filtered and lyophilised for total DNA isolation. The fungal DNA extraction was done with the Wizard Magnetic DNA Purification System for Food (Promega) with some modifications by halving the volume of the reaction, starting from 10 mg of lyophilised mycelium. The quality of genomic DNA was determined by agarose gel electrophoresis and quantification by means of a NanoDrop ND-1000 Spectrophotometer. Amplification of part of the β-tubulin gene (*benA*) was performed using the primers T1 (O'Donnell and Cigelnik 1997) and CYLTUB1R (Crous et al. 2004), for the Histone H3 region (*HIS3*) primers CYLH3F and CYLH3R (Crous et al. 2004) were used and for the translation elongation factor 1-α (*TEF-1a*) the primers EF1-728F (Carbone and Kohn 1999) and CylEF-R2 (Crous et al. 2004) were used. Amplification reactions and cycle conditions followed Crous et al. (2004), except that an annealing temperature of 58 °C was used for all reactions.

In addition, the ITS region of the six strains used in morphological and phytopathological studies was sequenced using standard conditions and primers ITS1/ITS4 (White et al. 1990). Although this locus could not successfully distinguish all species of *Ilyonectria*, this region was recently accepted as the universal fungal barcode region (Schoch et al. 2012).

Blast searches using the NCBI GenBank nucleotide database confirmed that the isolated fungus belonged to *Ilyonectria*, but without a 100 % match to any known species in the database. Representative sequences (Table 2) from previous publications (Schroers et al. 2008; Cabral et al. 2012a, b) were downloaded from the NCBI GenBank nucleotide database to act as a reference backbone for the phylogenetic analyses. The preliminary alignment of the three sequenced loci (*benA*, *HIS3*, *TEF-1a*) was performed using BioNumerics v. 5.1 (Applied Maths), with manual adjustment by eye where necessary. Based on previous publications (e.g. Cabral et al. 2012a, b), *Campylocarpon fasciculare* (CBS 112613) was used as outgroup and some representative strains of the *Ilyonectria radicicola* species complex were included in the analysis (Table 2). The phylogenetic analysis was conducted firstly on these individual locus alignments and subsequently the combined alignment of the three loci was analysed for inferring the organismal phylogeny. The combined alignment was created using the Muscle algorithm in MEGA v. 5 (Tamura et al. 2011). Each locus was first aligned separately and then concatenated in a super-gene alignment used to generate the phylogenetic tree. Phylogenetic analyses were performed in MEGA v. 5 using both Neighbour-Joining (NJ) (Saitou and Nei 1987) and Maximum Likelihood (ML) methods and the Tamura-Nei model (Tamura and Nei 1993). The best model substitution was calculated and the best option was found to be the Tamura 3-parameter model with Gamma distribution. A parsimony analysis of the aligned sequence data was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 100 random simple taxa additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees (limited to 1,000 trees) were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis and Bull 1993). Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated. MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings for each data partition. Based on the results of the MrModeltest, a model-optimised phylogenetic reconstruction was performed for the aligned combined

Table 2 "*Cylindrocarpon*", *Ilyonectria* and *Neonectria* isolates used in the phylogenetic analyses

Species	Strain number and status	GenBank accession no.				
		ITS	TUB	H3	EF	Host/substrate
"Cylindrocarpon" cylindroides	CBS 324.61, representative strain	JF735312	DQ789875	JF735599	JF735788	*Abies concolor*
"Cylindrocarpon" pauciseptatum	CBS 120171[a]	EF607089	EF607066	JF735587	JF735776	*Vitis* sp.
Ilyonectria liriodendri	CBS 110.81[a] of *C. liriodendri*	DQ178163	DQ178170	JF735507	JF735696	*Liriodendron tulipifera*, root
Ilyonectria lusitanica	CBS 129080	JF735296	JF735423	JF735570	JF735759	*Vitis vinifera*
Ilyonectria macrodidyma	CBS 112615, holotype of *C. macrodidymum*	AY677290	AY677233	JF735647	JF735836	*Vitis vinifera*
Ilyonectria mors-panacis	CBS 306.35[a] of *Ramularia mors-panacis*	JF735288	JF735414	JF735557	JF735746	*Panax quinquefolium*
Ilyonectria mors-panacis	CBS 124662[a] of *C. destructans* f.sp. *panacis*	JF735290	JF735416	JF735559	JF735748	*Panax ginseng*
Ilyonectria novozelandica	CBS 112593	AY 677281	AY677236	JF735631	JF735820	*Vitis vinifera*
Ilyonectria palmarum sp. nov.	BRA1	HF937429	HF922606	HF922618	HF922612	*Brahea armata*
	BRA2	HF937430	HF922607	HF922619	HF922613	*Brahea armata*
	HF3 = CBS 135754[a]	HF937431	HF922608	HF922620	HF922614	*Howea fosteriana*
	HF7	HF937432	HF922609	HF922621	HF922615	*Howea fosteriana*
	TP1	HF937433	HF922610	HF922622	HF922616	*Trachycarpus princeps*
	TP3	HF937434	HF922611	HF922623	HF922617	*Trachycarpus princeps*
Ilyonectria radicicola	CBS 264.65[a]	AY677273	AY677256	JF735506	JF735695	*Cyclamen persicum*
Ilyonectria robusta	CBS 308.35[a] of *Ramularia*	JF735264	JF735377	JF735518	JF735707	*Panax quinquefolium*
Ilyonectria rufa	CBS 137.37, authentic strain of *Coleomyces rufus*	AY677271	AY677251	JF735540	JF735729	Dune sand
Ilyonectria torresensis	CBS 113555	JF735350	AY677234	JF735661	JF735850	*Vitis* sp.
Neonectria ditissima	CBS 226.31, authentic strain of *C. wilkommii*	JF735309	DQ789869	JF735594	JF735783	*Fagus sylvatica*
Neonectria ditissima	CBS 835.97, representative strain of *N. galligena*	JF735310	DQ789880	JF735595	JF735784	*Salix cinerea*
Neonectria major	CBS 240.29[a]	JF735308	DQ789872	JF735593	JF735782	*Alnus incana*
Neonectria neomacrospora	CBS 118984, representative strain	JF735311	DQ789882	JF735598	JF735787	*Arceuthobium tsugense*
Neonectria ramularia	CBS 151.29, authentic strain of *C. obtusiusculum* (= *C. magnusianum*)	JF735313	JF735438	JF735602	JF735791	*Malus sylvestris*

[a] Ex-type strain

data set to determine species relationships using MrBayes v. 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The heating parameter was set at 0.25 and the Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies came below 0.01. Trees were saved each 1,000 generations and the resulting phylogenetic tree was printed with Geneious v. 5.5.4 (Drummond et al. 2011). New sequences were lodged in GenBank and the alignment and phylogenetic tree in TreeBASE (www.treebase.org).

Pathogenicity

The pathogenicity of four representative isolates (HF1, BRA1, BRA5 and TP1) was evaluated on potted, 1- and 3-year-old plants of *H. forsteriana, B. armata*, *B. edulis* and *T. princeps*. Plants were respectively grown in a growth chamber as well as in a nursery. Trials were

conducted on 30 plants for each host species/age. The same number of plants was used as control. All 1-year-old plants were potted in 0.5–l containers in a 1:3:1 Canadian sphagnum peat, sand, and perlite and were inoculated by soil drench at the base of each plant (10 ml/pot) with conidial suspension ($1–2.5\times10^5$ conidia ml^{-1}) obtained from cultures grown on PDA dishes and incubated at 25 °C for 2 weeks. A mixture of conidia and mycelia as inoculum was prepared for each isolate by flooding the dishes with sterile water and rubbing the colony surface with a sterile loop. Sterile water was applied to control plants. Thirty 3-year-old plants were potted in 8-l containers in a 1:3:1 Canadian sphagnum peat, sand, and perlite and were inoculated with one mycelial plug obtained from 14-day-old colonies growing on PDA and applied to a 6 mm diam crown wound. The same number of plants was inoculated without wounding. Sterile agar plugs were used in control plants. Following inoculation, the crowns were wrapped with Parafilm and all plants were covered with plastic bags for 48 h. One-year-old plants were maintained in a growth chamber at 25±1 °C, while all 3-year-old plants were cultivated in a nursery. All plants were irrigated 2–4 times during every week and were fertilised according to farm use every 30 days with 2 or 20 g/pot of complex NPK fertilizer Nitrophoska® special (BASF). After 4 and 8 months, plants were examined for disease symptoms.

Results

Field survey and isolation

During the survey, bending and basal stem rot were observed in all nurseries where *Brahea* spp. were cultivated (Fig. 1). Symptoms were detected in four nurseries on approximately 6400 of 2- to 8-year-old potted *B. armata* and on 1100 *B. edulis* plants of the same age. In these nurseries the disease incidence varied from 22.80 to 40 % on *B. armata* to 18.1–35.2 % on *B. edulis* plants. Bending and basal stem rot were also observed on 60 of 3-year-old *H. forsteriana* plants in one nursery and on 100 of 3-year-old *T. princeps* plants in another nursery (Fig. 1). On these hosts the disease incidence varied from 0.3 (*H. forsteriana*) to 10 % (*T. princeps*).

No symptoms were detected on *T. fortunei*, *C. humilis*, *P. canariensis*, *P. robelenii*, *A. romanzoffianum* and *W. robusta* plants. A cylindrocarpon-like species was consistently isolated from diseased ornamental palms tissues. Species of *Ganoderma, Phytophthora* and *Thielaviopsis* were not found associated with symptomatic tissues, or recovered on standard and selective media.

A total of 40 cylindrocarpon-like isolates were collected from diseased palm hosts. The majority of the isolates (22) were associated with basal stem rot of *Brahea* spp., nine from *T. princeps*, and nine from *H. forsteriana* (Table 1).

Morphology

Ilyonectria palmarum G. Polizzi, G. Perrone & Crous, sp. nov.

MycoBank MB804338; Fig. 2

Perithecia formed homothallically in vitro, solitary or in groups of 2–3, developing directly on the SNA agar surface, ovoid to obpyriform, red, becoming purple-red in 3 % KOH (positive colour reaction), finely warted, 250–350 μm diam, up to 300–350 μm high; without recognisable stroma; perithecial wall consisting of two poorly distinguishable regions; outer region 10–15 μm thick, composed of 3–4 layers of angular to subglobose cells, 7–20×5–8 μm; cell walls up to 1 μm thick; inner region around 5 μm thick, composed of cells that are flat in transverse optical section and angular to oval in subsurface optical face view; perithecial warts consisting of globose to subglobose cells, 15–25×10–20 μm, that have walls up to 1.5 μm thick. *Asci* subcylindrical to narrowly clavate, 40–70×5–8 μm, 8-spored; apex truncate to bluntly rounded, with a visible ring. *Ascospores* divided into two cells of equal size, ellipsoidal, tapering towards both ends, smooth to finely warted, guttulate, (9–)10(−11)×3(−3.5) μm. *Conidiophores* simple or complex, sporodochial. Simple conidiophores arising laterally or terminally from the aerial mycelium or erect, arising from the agar surface, solitary to loosely aggregated, unbranched or sparsely branched, 1–4-septate, rarely consisting only of the phialide, 50–170 μm long; phialides monophialidic, cylindrical, slightly tapering toward the base, 50–70×2–3 μm, 2.5–3 μm near aperture. Complex, sporodochial conidiophores aggregated in pionnote sporodochia, repeatedly, irregularly branched; phialides cylindrical but slightly tapering towards the tip, 25–50×2–3 μm, 1.5–2 μm near the aperture. *Micro-* and *macroconidia* present on both types of conidiophores. *Macroconidia* (2–)3-septate, straight or sometimes slightly curved,

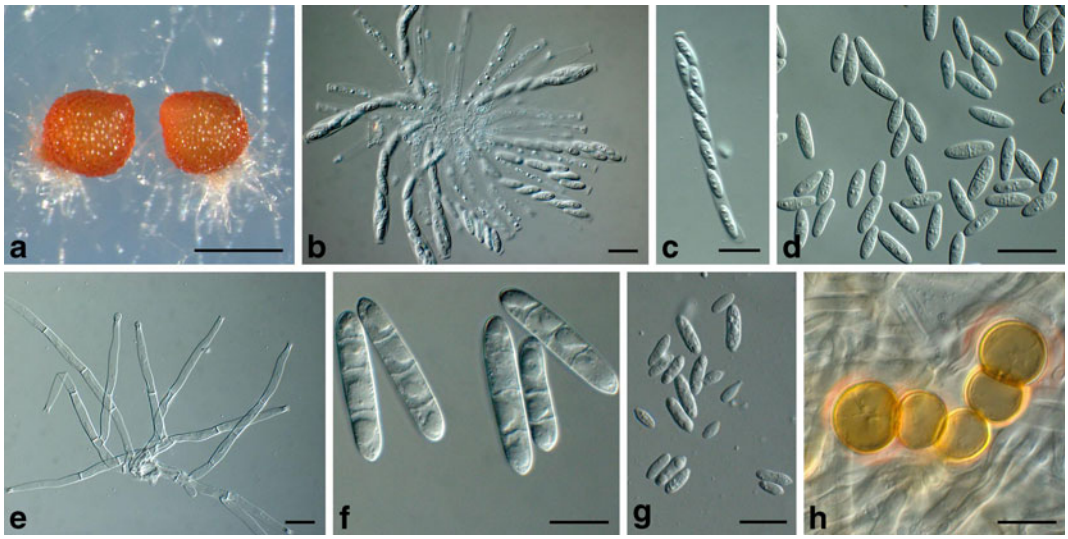


Fig. 2 *Ilyonectria palmarum* (CPC 22087). **a** Perithecia developing on synthetic nutrient-poor agar. **b**–**c** Asci. **d** Ascospores. **e** Conidiophores with phialides. **f** Three-septate macroconidia. **g** Microconidia. **h** Chlamydospores. Scale bars: *A*=300 μm, all others = 10 μm

cylindrical or typically minutely widening towards the tip, therefore appearing somewhat clavate, mostly with a visible, slightly laterally displaced hilum, (25–)32–37(−39)×(4–)5(−6) μm. *Microconidia* common on SNA, 0–1-septate, ellipsoidal to ovoidal or subcylindrical, more or less straight, with a clearly laterally displaced hilum; aseptate microconidia (6–)8–9(−10)×2.5–3(−4) μm; 1-septate microconidia (10–)11–13(−15)×(3–)3.5(−4) μm. *Conidia* formed in heads on simple conidiophores or as unpigmented masses on simple as well as complex conidiophores. *Chlamydospores* mostly in short, intercalary chains, golden-brown, globose to globose-ellipsoid, 8–15×9–12 μm.

Culture characteristics Covering surface of PDA dish after 2 week at 25 °C; surface was appressed, dense, lacking zonation, and with sparse aerial mycelium; centre dark brick, outer zone cinnamon, reverse dark brick in centre, brick in outer zone (Rayner 1970).

Cardinal temperatures for growth After 7 days at 4 °C all isolates only grew on the agar plug, while no growth was observed at 35 °C. Optimal growth occurred at 25 °C, with colonies reaching 30–62 mm diam.

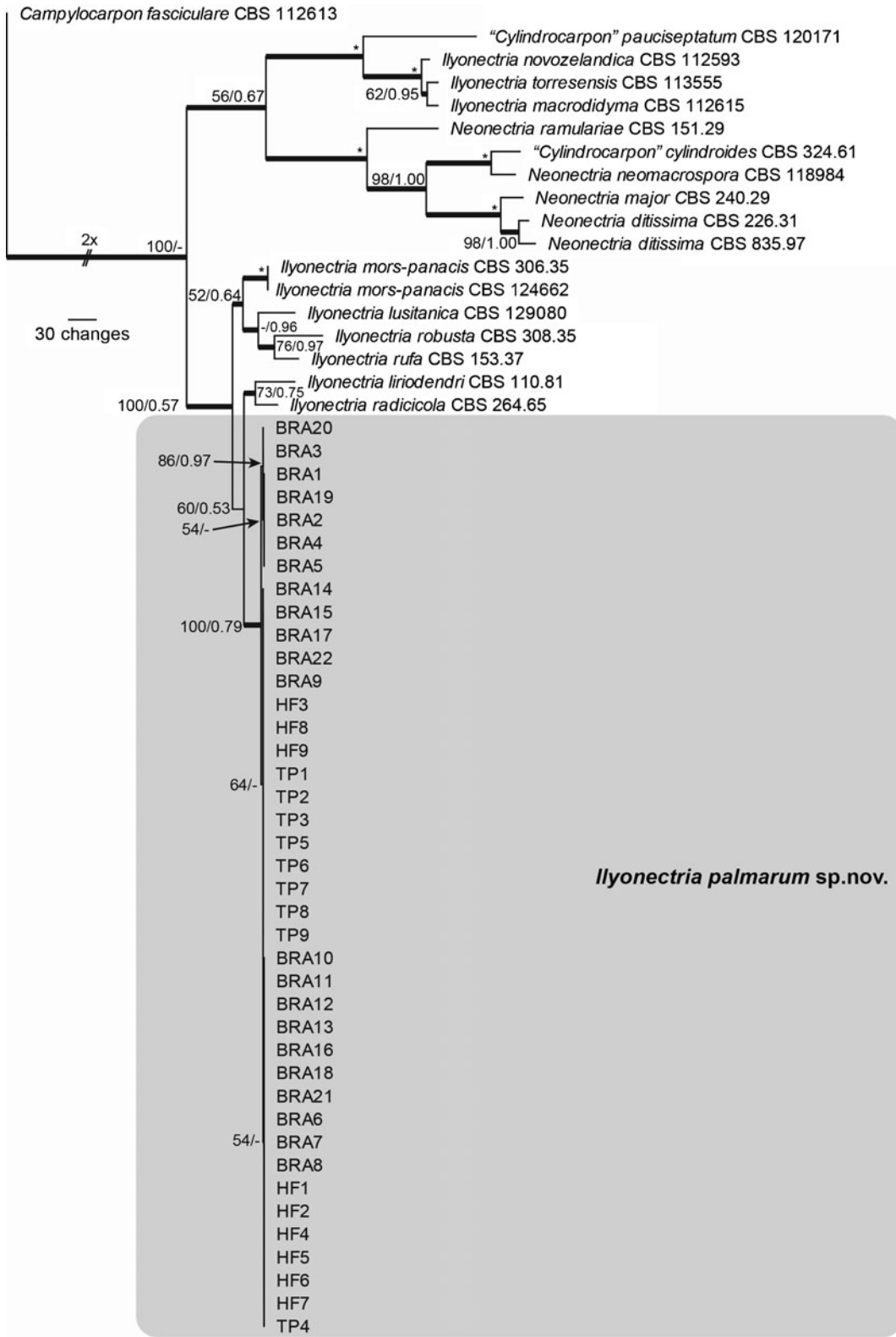
***Typus*: Italy**, Sicily, Catania province, Aci Castello, basal stem rot on *Howea forsteriana*, July 2011, G. Polizzi, **holotype** CBS H-135754, culture ex-type DiGeSA-HF3 = CPC 22087 = CBS 135754.

Additional specimens examined Italy, Sicily, Catania province, Praiola, Giarre, Praiola, Giarre, basal stem rot on *Brahea armata*, Sept. 2010, G. Polizzi, DiGeSA-BRA1 = CPC 22085 = CBS 135755; DiGeSA-BRA2 = CPC 22086 = CBS 135228; Aci Castello, basal stem rot on *Howea forsteriana*, July 2011, G. Polizzi, DiGeSA-HF7 = CPC 22088 = CBS 135753; Carruba, Giarre, basal stem rot on *Trachycarpus princeps*, Feb. 2012, G. Polizzi, DiGeSA-TP1 = CPC 22089 = CBS 135229; Carruba, Giarre, basal stem rot on *Trachycarpus princeps*, Feb. 2012, G. Polizzi, DiGeSA-TP3 = CPC 22090 = CBS 135756.

Molecular identification

We obtained amplicons of approximately 560 bases for *benA*, 510 for *HIS3* and 460 for *TEF-1α* for all isolates. The consensus sequences from these amplicons were used in the combined alignment.

The manually adjusted combined (*benA*, *TEF-1α* and *HIS3*) alignment contained 58 taxa (including the outgroup sequence) and, of the 1,550 characters (516, 498 and 536 for *benA*, *TEF-1α* and *HIS3*, respectively) used in the phylogenetic analysis, 511 (152, 189 and 170 for *benA*, *TEF-1α* and *HIS3*, respectively) were parsimony-informative, 218 (75, 96 and 47 for *benA*, *TEF-1α* and *HIS3*, respectively) were variable and parsimony-uninformative, and 821 (289, 213 and 319 for *benA*, *TEF-1α* and *HIS3*, respectively) were constant.


Campylocarpon fasciculare CBS 112613
"Cylindrocarpon" pauciseptatum CBS 120171
Ilyonectria novozelandica CBS 112593
Ilyonectria torresensis CBS 113555
Ilyonectria macrodidyma CBS 112615
Neonectria ramulariae CBS 151.29
"Cylindrocarpon" cylindroides CBS 324.61
Neonectria neomacrospora CBS 118984
Neonectria major CBS 240.29
Neonectria ditissima CBS 226.31
Neonectria ditissima CBS 835.97
Ilyonectria mors-panacis CBS 306.35
Ilyonectria mors-panacis CBS 124662
Ilyonectria lusitanica CBS 129080
Ilyonectria robusta CBS 308.35
Ilyonectria rufa CBS 153.37
Ilyonectria liriodendri CBS 110.81
Ilyonectria radicicola CBS 264.65
56/0.67
62/0.95
98/1.00
98/1.00
2x
100/-
52/0.64
-/0.96
76/0.97
73/0.75
100/0.57
30 changes
86/0.97
60/0.53
54/-
100/0.79
64/-
54/-
BRA20
BRA3
BRA1
BRA19
BRA2
BRA4
BRA5
BRA14
BRA15
BRA17
BRA22
BRA9
HF3
HF8
HF9
TP1
TP2
TP3
TP5
TP6
TP7
TP8
TP9
BRA10
BRA11
BRA12
BRA13
BRA16
BRA18
BRA21
BRA6
BRA7
BRA8
HF1
HF2
HF4
HF5
HF6
HF7
TP4
Ilyonectria palmarum sp.nov.

◀ **Fig. 3** The first of five equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment using PAUP v. 4.0b10. Strains from the novel species are included in the *coloured box*. The scale bar shows 30 changes, and bootstrap support values from 1000 replicates and Bayesian posterior probability values are shown at the nodes. An *asterisk* ("*") indicates nodes with bootstrap support values of 100 % and Bayesian posterior probability values of 1. *Thickened lines* indicate the strict consensus branches and the tree was rooted to *Campylocarpon fasciculare* CBS 112613 (*benA* = AY677221, *TEF-1a* = JF735691, *HIS3* = JF735502)

The percentage of variable sites and parsimony informative sites for each locus differ, the *TEF-1α* sequences have the highest percentage (57.23 %) of variable and parsimony informative sites, and *HIS3* have the lowest variability (40.49 %). Five equally most parsimonious tree were saved from the heuristic search and the first of these is shown in Fig. 3 (TL = 1,632, CI = 0.718, RI = 0.829, RC = 0.595). Based on the results of MrModeltest, the following priors were set in MrBayes for the different partitions: all partitions had dirichlet base frequencies

Fig. 4 Symptoms induced on containerised palms by *Ilyonectria palmarum* sp. nov. isolates 8 months after plugs inoculations. **a**–**c**. Dry basal stem rot and breakage of trunk on *Brahea armata* plants. **d**. Trunk bending and emission of new leaves below the decayed tissues on *B. edulis* plants. **e**–**g**. Basal stem rot, trunk bending and tissue proliferation on *Howea forsteriana* plants

and, per partition, the HKY85 model with gamma-distributed rates (for *benA*), the GTR model with gamma-distributed rates (for *TEF-1α*), or the GTR model with inverse gamma-distributed rates (for *HIS3*). The alignment contained 622 unique site patterns (188, 231, and 203 for *benA*, *TEF-1α* and *HIS3*, respectively). The Bayesian analysis lasted 1,995,000 generations and the consensus trees and posterior probabilities were calculated from the 2,994 trees left after discarding the first 25 % for burn-in. The phylogenetic tree obtained from the Bayesian analysis has the same overall topology as that obtained from the parsimony analysis (data not shown; posterior probability values superimposed on Fig. 3). In addition, the Maximum Likelihood phylogenetic tree obtained in MEGA 5 software had the highest log likelihood of (−6807.4661) with a total of 1,360 positions in the final dataset (see Supplementary Fig S3).

Phylogenetic analyses were also conducted on the individual data partitions (data not shown) and the resulting trees contained a similar overall topology, fulfilling the requirements of genealogical concordance phylogenetic species recognition (GCPSR, Taylor et al. 2000).

Pathogenicity

All *Ilyonectria* isolates tested were pathogenic on the host species inoculated, and produced identical symptoms to those observed in the nurseries (Fig. 4). After 4 months, trunk bending was only detected on the 3-year-old plants with wounds. After 8 months, plants showed basal stem rot and trunk bending. The root rot was observed occasionally in 1-year-old plants of *H. forsteriana*. Plant death was however observed on

Table 3 Pathogenicity of *Ilyonectria palmarum* isolates on ornamental palms 4 and 8 months after inoculation

Palm species/age	Isolate	Inoculation method[a]	Number of symptomatic plants				
			After 4 months[b]		After 8 months[b]		
			TB	DP	BSR	TB	DP
Howea forsteriana/1 year	HF3	CS/NW	0	1	2	0	2
Control	–	SW/NW	0	0	0	0	0
Brahea armata/1 year	BRA1	CS/NW	0	1	1	0	2
Control	–	SW/NW	0	0	0	0	0
Brahea edulis/1 year	BRA5	CS/NW	0	2	1	0	2
Control	–	SW/NW	0	0	0	0	0
B. armata/3 years	BRA1	AFP/NW	0	0	0	0	0
Control		AP/NW	0	0	0	0	0
B. edulis/3 years	BRA5	AFP/NW	0	0	0	0	0
Control		AP/NW	0	0	0	0	0
H. forsteriana/3 years	HF3	AFP/W	2	0	28	5	3
Control	–	AP/W	0	0	0	0	0
B. armata/3 years	BRA1	AFP/W	2	1	28	6	3
Control	–	AP/W	0	0	0	0	0
B. edulis/3 years	BRA5	AFP/W	3	2	26	6	4
Control	–	AP/W	0	0	0	0	0
Trachycarpus princeps/3 years	TP1	AFP/W	2	1	22	5	3
Control	–	AP/W	0	0	0	0	0

Thirty plants were inoculated for each species/age

[a] *CS/NW* conidial suspension drench applied with no wound; *SW/NW* sterile water drench applied with no wound; *AFP/NW* agar fungal plug applied with no wound; *AP/NW* agar plug applied with no wound; *AFP/W* agar fungal plug applied with wound; *AP/W* agar plug applied with wound

[b] *BSR* basal stem rot; *TB* trunk bending; *DP* death of plant

plants of *H. forsteriana* and *Brahea* spp., with and without wounds. Basal stem rot and trunk bending were observed on all host species when the plants were inoculated with agar fungal plug applied with wound (Table 3). Isolations from the symptomatic plants consistently yielded the test fungi. All uninoculated control plants remained healthy.

Discussion

Basal stem or trunk rot of palms is normally caused by different fungal species including *Ganoderma* and *Phytophthora* spp., and *T. paradoxa*. In our study, a new and widespread dry basal stem rot was detected on potted *B. armata*, *B. edulis*, *H. forsteriana*, and *T. princeps* ornamental palms cultivated in different nurseries in eastern Sicily. A cylindrocarpon-like asexual morph was consistently obtained from symptomatic tissues of these palms, while *Ganoderma* spp., *Phytophthora* spp., and *T. paradoxa* were not detected in standard or selective media.

According to the morphological classification of isolates associated with trunk rot of palms, this species has microconidia and chlamydospores, and thus belongs to "*Cylindrocarpon* group 3" sensu Booth (1966). Species in this group, along with their neonectria-like sexual morphs were recently relocated to the genus *Ilyonectria* (Chaverri et al. 2011). Several important plant pathogenic species inducing black foot rot of a range of hosts belong to this genus (Seifert et al. 2003; Halleen et al. 2004, 2006; Chaverri et al. 2011; Cabral et al. 2012a, b). Based on the multigene DNA analysis conducted of known species, isolates from palm were shown to represent a novel species, described here as *I. palmarum*.

Ilyonectria palmarum was pathogenic to the different host species inoculated, and produced identical symptoms to those observed in nurseries, thus confirming its role as causal agent of the new basal rot of containerised palms in Italy. On the basis of the disease incidence and severity observed in several nurseries we believe that this disease poses a serious threat, especially to the containerised field-grown species of *Brahea*. The reasons that promoted this unprecedented occurrence of *I. palmarum* associated with a basal stem rot are unknown. The containerised palm production could have a role in promoting infections because the palms are frequently stressed, remain containerised throughout production, and several wounds could be incurred during transplanting. The predominant potting component in field-grown palm nurseries in most of the eastern Sicily is autochtone volcanic soil mixed with peat and perlite or vermiculite to assure plant stability and proper development. Thus, the use of non-disinfected volcanic soil could represent a possible source of pathogen inoculum.

Species of *Ilyonectria* are commonly found in soil, and cause root diseases on a range of diverse hosts worldwide (Sánchez et al. 2002; Seifert et al. 2003; Halleen et al. 2006; Alaniz et al. 2007, 2009; Agustí-Brisach et al. 2011; Dart and Weeda 2011; Petit et al. 2011; Cabral et al. 2012a, b; Özben et al. 2012; Úrbez-Torres et al. 2012; Vitale et al. 2012; Erper et al. 2013). It is interesting to note, however, that *Ilyonectria* spp. were not previously reported as causal agents of palm diseases. Previously, Halleen et al. (2006) identified a single strain (IMI 313237) clustering with the ex-type culture of *I. radicicola* (CBS 264.65) and isolated from arecoid palm (Seifert et al. 2003), but no data about the pathogenicity of this isolate is reported in the literature. As far as we could establish, this is the first confirmed report of basal stem rot and stem bending caused by a *Ilyonectria* species on palms.

Acknowledgments This work was supported by MIUR project number PON01_01611 (SO.PRO.ME: Sustainable production of potted plants in Mediterranean environment"). The authors would like to thank Gaetano Stea for his valuable technical help in DNA sequencing.

References

Agustí-Brisach, C., Gramaje, D., León, M., García-Jiménez, J., & Armengol, J. (2011). Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens. *Plant Disease, 95*, 803–810.

Alaniz, S., León, M., Vincent, 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.

Alaniz, S., Armengol, J., León, M., García-Jiménez, J., & Abad-Campos, P. (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.

Ariffin, D., & Idris, A. S. (1991). *A selective medium for the isolation of Ganoderma from diseased tissue*. Proceedings of the 1991 PIPOC International Palm Oil Conference-Progress, Prospects and Challenges Towards the 21st Century (Model I-Agriculture), MPOB, Malaysia, pp. 517–519.

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

Cabral, A., Groenewald, J. Z., Rego, C., Oliveira, H., & Crous, P. W. (2012a). Cylindrocarpon root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. *Mycological Progress, 11*, 655–688.

Cabral, A., Rego, C., Nascimento, T., Oliveira, H., Groenewald, J. Z., & Crous, P. W. (2012b). Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. *Fungal Biology, 116*, 62–80.

Cacciola, S. O., Pane, A., Faedda, R., Rizza, C., Badalà, F., & Magnano di San Lio, G. (2011). Bud and root rot of windmill palm (*Trachycarpus fortunei*) caused by simultaneous infections of *Phytophthora palmivora* and *P. nicotianae* in Sicily. *Plant Disease, 95*, 769.

Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia, 91*, 553–556.

Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A. Y., & Samuels, G. J. (2011). Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology, 68*, 57–78.

Chong, K. P., Lum, M. S., Foong, C. P., Wong, C. M. V. L., Atong, M., & Rossall, S. (2011). First identification of *Ganoderma boninense* isolated from Sabah based on PCR and sequence homology. *African Journal of Biotechnology, 10*, 14718–14723.

Crous, P. W., Groenewald, J. Z., Risède, J. M., Simoneau, P., & Hywel-Jones, N. (2004). *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology, 50*, 415–430.

Crous, P. W., Verkleij, G. J. M., Groenewald, J. Z., & Samson, R. A. (Eds.). (2009). *Fungal biodiversity. CBS Laboratory Manual Series 1*. Utrecht: Centraalbureau voor Schimmelcultures.

Dart, N. L., & Weeda, S. M. (2011). First report of *Cylindrocarpon macrodidymum* on *Acer palmatum* in Virginia. *Plant Disease, 95*, 1592.

Downer, A. J., Uchida, J. Y., Hodel, D. R., & Elliott, M. L. (2009). Lethal palm diseases common in the United States. *HortTechnology, 19*, 710–716.

Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., et al. (2011). Geneious v5.4. from http://www.geneious.com/.

Elliott, M. L. (2004). Ganoderma butt rot. In M. L. Elliott, T. K. Broschat, J. Y. Uchida, & G. W. Simone (Eds.), *Compendium of ornamental palm diseases and disorders* (pp. 22–25). Saint Paul: APS Press.

Elliott, M. L. (2006). *Thielaviopsis trunk rot of palm*. University of Florida Institute of Food and Agricultural Science, Fact Sheet. PP-219.

Elliott, M. L., & Broschat, T. K. (2001). Observations and pathogenicity experiments on *Ganoderma zonatum* in Florida. *Palms, 45*, 62–72.

Erper, I., Agustí-Brisach, C., Tunali, B., & Armengol, J. (2013). Characterization of root rot disease of kiwifruit in the BlacK Sea region of Turkey. *European Journal of Plant Pathology, 136*, 291–300.

Halleen, F., Schroers, H. J., Groenewald, J. Z., & Crous, P. W. (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., Schroers, H. J., Groenewald, J. Z., Rego, C., Oliveira, H., & Crous, P. W. (2006). *Neonectria liriodendri* sp. nov., the main causal agent of black foot disease of grapevines. *Studies in Mycology, 55*, 227–234.

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology, 42*, 182–192.

Huelsenbeck, J. P., & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*, 754–755.

Jeffers, S. N., & Martin, S. B. (1986). Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease, 70*, 1038–1043.

Miller, R. N. G., Holderness, M., Bridge, P. D., Chung, G. F., & Zakaria, M. H. (1999). Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology, 48*, 595–603.

Nylander, J. A. A. (2004). *MrModeltest 2.0*. Program distributed by the author. Uppsala, Sweden: Uppsala University.

O'Donnell, K., & Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution, 7*, 103–116.

Özben, S., Demirci, F., Değirmenci, K., & Uzunok, S. (2012). First report of *Cylindrocarpon macrodidymum* associated with black foot disease of grapevine in Turkey. *Plant Disease, 96*, 762.

Petit, E., Barriault, E., Baumgartner, K., Wilcox, W. F., & Rolshausen, P. E. (2011). *Cylindrocarpon* species associated with black-foot of grapevine in northeastern United States and southeastern Canada. *American Journal of Enology and Viticulture, 62*, 177–183.

Polizzi, G., Castello, I., Vitale, A., Catara, V., & Tamburino, V. (2006). First report of Thielaviopsis trunk rot of date palm in Italy. *Plant Disease, 90*, 972.

Polizzi, G., Castello, I., Aiello, A., & Vitale, A. (2007). First report of stem bleeding and trunk rot of kentia palm caused by *Thielaviopsis paradoxa* in Italy. *Plant Disease, 91*, 1057.

Rayner, R. W. (1970). *A mycological colour chart*. CMI and British Mycological Society. Surrey, England: Kew.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*, 1572–1574.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406–425.

Sánchez, M. E., Lora, F., & Trapero, A. (2002). First report of *Cylindrocarpon destructans* as a root pathogen of Mediterranean *Quercus* species in Spain. *Plant Disease, 86*, 693.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 6241–6246.

Schroers, H. J., Žerjav, M., Munda, A., Halleen, F., & Crous, P. W. (2008). Cylindrocarpon pauciseptatum sp. nov. with notes on Cylindrocarpon species with wide, predominantly 3-septate macroconidia. *Mycological Research, 112*, 82–92.

Seifert, K. A., McMullen, C. R., Yee, D., Reeleder, R. D., & Dobinson, K. F. (2003). Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus

Cylindrocarpon destructans. *Phytopathology, 93*, 1533–1542.

Simone, G. W. (2004). Thielaviopsis diseases (black scorch, stem bleeding, dry basal rot, trunk rot, heart rot). In M. L. Elliott, T. K. Broschat, J. Y. Uchida, & G. W. Simone (Eds.), *Compendium of ornamental palm diseases and disorders* (pp. 37–38). Saint Paul: APS Press.

Swofford, D. L. (2003). *PAUP*. Phylogenetic analysis using parsimony (*and their methods). Version 4*. Sunderland: Sinauer Associates.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution, 10*, 512–526.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., & Geiser, D. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology, 31*, 21–32.

Uchida, J. Y. (2004). Phytophthora diseases. In M. L. Elliott, T. K. Broschat, J. Y. Uchida, & G. W. Simone (Eds.), *Compendium of ornamental palm diseases and disorders* (pp. 29–32). Saint Paul: APS Press.

Úrbez-Torres, J. R., Peduto, F., & Gubler, W. D. (2012). First report of *Ilyonectria macrodidyma* causing root rot of olive trees (*Olea europaea*) in California. *Plant Disease, 96*, 1378.

Vitale, A., Aiello, D., Guarnaccia, V., Perrone, G., Stea, G., & Polizzi, G. (2012). First outbreak of root rot caused by *Neonectria* (*Ilyonectria*) *macrodidyma* on avocado (*Persea americana*) in Europe. *Journal of Phytopathology, 160*, 156–159.

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). New York: Academic.

Wong, L. C., Bong, C. F. J., & Idris, A. S. (2012). *Ganoderma* species associated with basal stem rot disease of oil palm. *American Journal of Applied Sciences, 9*, 879–885.