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ARTICLE

Pleiocarpon gen. nov. and a new species of *Ilyonectria* causing basal rot of *Strelitzia reginae* in Italy

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Abstract: During 2015, a new basal rot disease was observed on potted plants of *Strelitzia reginae* in an ornamental nursery located in eastern Sicily. Isolations from symptomatic parts of these diseased plants consistently yielded cylindrocarpon-like isolates. Multigene analyses of the partial gene regions of 28S large subunit nrDNA, β-tubulin, histone H3, translation elongation factor 1-alpha, internal transcribed spacer region and intervening 5.8S nrRNA gene, and RNA polymerase II second largest subunit genes, supported by morphological characters supported the recognition of a new genus, *Pleiocarpon* based on *P. strelitziae* sp. nov., and a new *Ilyonectria* species, described here as *I. strelitziae* sp. nov. The pathogenicity of both *I. strelitziae* and *P. strelitziae* were confirmed on young plants cultivated under controlled conditions in a growth chamber. Both cylindrocarpon-like fungi were pathogenic to *S. reginae* and reproduced symptoms similar to those observed in the nursery. Of the two species, *P. strelitziae* was more aggressive than *I. strelitziae,* resulting in the death of all inoculated plants.

Key words:
Nectriaceae
multigene phylogeny
pathogenicity
taxonomy

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INTRODUCTION

Strelitzia reginae, also well-known as “bird of paradise”, is one of the most commercially cultivated species of *Strelitzia* in the world (Karsten 2009). This species was first introduced into Europe from South Africa in 1770 for its unique flowers, which are characterized by vivid orange and bright purple or blue inflorescens, making it a highly sought-after cut flower crop (Van Jaarsveld 2008, Xaba 2011). This plant is also widely used in landscaping as a focal point for many European gardens (Xaba 2011). However, commercial cultivation of *S. reginae*, as cut flowers or as pot plants, is limited by fungal diseases, which are poorly reported in the literature.

In Hawaii, a *Pythium* sp. has been reported as causal agent of root rot on *S. reginae*, and a similar disease was reported in Egypt and Florida associated with *Rhizoctonia solani* and *Fusarium* spp. (Raabe *et al*. 1981, Alfenas *et al*. 1984, Hilal & Helmy 1998). In Italy, the most commonly reported fungal diseases of *S. reginae* are root and foot rot caused by *Phytophthora nicotianae* (Frisullo *et al*. 1987, Luongo *et al*. 2010), wilting associated with root rot caused by “*Cylindrocarpon” destructans* (Grasso & Cutuli 1972) and *Armillaria mellea* (Davino 1984), and southern blight caused by *Sclerotium rolfsii* (Polizzi *et al*. 2007).

During 2015, a new basal stem rot was detected on approximately 20 % of 20 000 potted *S. reginae* plants in a commercial nursery in Carrubba, Riposto (Catania province, eastern Sicily, Italy). The diseased plants displayed a dry rot of the basal stem which resulted in the detachment of the roots from the stem. In addition, symptoms of general wilting and rot of internal foliage were observed on the affected plants. The aims of this study were, therefore, to identify the pathogen(s) associated with the disease *via* morphological and molecular characterization, and to verify the pathogenicity of the organism(s) associated with these disease symptoms.

MATERIALS AND METHODS

Field survey and isolation

During 2015, a survey undertaken in an ornamental nursery in eastern Sicily led to the discovery of *Strelizia reginae* plants displaying symptoms of dry basal stem rot (Fig. 1). Some affected plants were randomly selected and brought to the laboratory for further analyses. The symptomatic stem tissues were surface-sterilized with 1.2 % (v/v) sodium hypochlorite for 2 min, rinsed three times in sterile distilled water and dried on sterile absorbent paper. Isolations were done by transferring fragments of symptomatic and the bordering healthy plant tissue onto potato dextrose agar (PDA, Oxoid™ Lda, UK) plates (amended with streptomycin sulphate at 100 ppm) and *Phytophthora* selective medium PARPH (Jeffers

Fig. 1. Dry basal rot symptoms of *Strelitzia reginae* observed in the nursery. **A–C.** Wilting and dying *S. reginae* plants. **D.** Dry basal stem rot. **E.** Rot of internal leaf.

& Martin 1986). The plates were incubated at 25 °C and examined daily for 1 wk. From these primary isolations, single-conidial isolates were derived and maintained on PDA slants for further study.

DNA isolation, sequencing and phylogenetic analyses

Total genomic DNA was extracted from 7-d-old fungal strains grown on PDA at room temperature (20–24 ºC) using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI) according to the manufacturer's protocol. Six genomic gene regions were targeted for amplification and sequencing using the primer pairs and protocols described by Lombard *et al*. (2015): 28S large subunit (LSU) nrDNA, internal transcribed spacer regions and intervening 5.8S nrRNA (ITS), translation elongation factor 1-alpha (*tef1*), histone H3 (*his3*), β-tubulin (*tub2*), and the RNA polymerase II second largest subunit (*rpb2*) gene regions.

Amplicons were sequenced in both directions using the same primers used for amplification and the BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA) following the protocol provided by the manufacturer. Sequences were determined on an Applied Biosystems 3730 DNA Analyzer (Life Technologies, Carlsbad, CA). The generated sequences were analysed and consensus sequences were determined using Seqman (DNAStar, Madison, WI). All sequences were manually corrected and the arrangement of nucleotides in ambiguous positions corrected by comparisons of the sequences generated from both the forward and reverse primers. In addition to the sequences generated in this study, other sequences of closely related *Nectriaceae* were obtained from NCBI's GenBank nucleotide database and added to the sequence datasets generated in this study. The sequences for each locus were aligned using MAFFT v. 7 (Katoh & Standley 2013). The alignments were manually checked using MEGA v. 7 (Kumar *et al.* 2015) and improved where necessary. Novel sequences were lodged in GenBank (Table 1), and the alignments and phylogenetic trees in TreeBASE (S20598). Congruency of the six loci was tested using the 70 % reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996) following the protocol of Lombard *et al.* (2015) for each locus.

Phylogenetic analyses were based on Bayesian inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP). For both BI and ML, the evolutionary model for each partition was determined using MrModeltest (Nylander 2004) and incorporated into the analyses. For the BI analysis, MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003) was used to generate phylogenetic trees under the optimal model per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set to 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies decreased below 0.01 with trees saved each 1 000 generations. The first 25 % of saved trees were discarded as "burn-in" and posterior probabilities determined from the remaining trees.

The ML analysis was made with RAxML (randomized axelerated [*sic*] maximum likelihood for high performance computing; Stamatakis 2014) through the CIPRES website (http://www.phylo.org) to obtain a second measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) analysis with the bootstrap replicates automatically determined by the software.

The MP analysis was carried out with PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 1 000 random sequence-additions. Tree bisection-reconnection was implemented, with the branch swapping option set on "best tree" only. All characters were weighted equally and alignment gaps were treated as "fifth state". Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). The bootstrap support analysis was based on 1 000 replications.

Taxonomy

Axenic cultures were grown on synthetic nutrient-poor agar (SNA; Nirenburg 1981) amended with 1-cm^2 sterile filter paper and carnation leaf pieces, and on PDA as described by Cabral *et al.* (2012a). Gross morphological characteristics were studied by mounting the fungal structures in 85 % lactic acid and 30 measurements were made for all taxonomically informative characters at ×1000 magnification using a

Table 1. Strains included in the phylogenetic analyses.

Species	Isolate nr.[1]	Substrate	Locality	GenBank Accession no.[2]					
				ITS	LSU	*his3*	*rpb2*	*tef1*	*tub2*
Campylocarpon fasciculare	**CBS 112613**; CPC 3970	*Vitis vinifera*	South Africa	AY677301	HM364313	–	–	JF735691	AY677221
C. pseudofasciculare	**CBS 112679**; CPC 5472	*V. vinifera*	South Africa	AY677306	HM364314	–	–	JF735692	AY677214
Cinnamomeonectria cinnamomea	CBS 136783; PC 1222	Bark	Brazil	KJ021998	KJ022049	–	–	KJ022376	KJ022344
	IMI 325248; G.J.S. 86-117	Bark of living liana	French Guiana	KJ022010	KJ022072	–	–	KJ022393	KJ022341
Cylindrocarpostylus gregarius	**CBS 101072**	*Hylurgops palliatus*	Germany	KM231747	JQ666084	–	–	KM231870	KM232005
	CBS 101073	*Pinus sylvestris*	Germany	KM231748	JQ666083	–	–	KM231871	KM232006
Cylindrodendrum album	**CBS 301.83**; ATCC 46842; IMI 255534	*Fucus distichus*	Canada	KM231764	KM231626	–	–	KM231889	KM232021
	CBS 110655	Soil	The Netherlands	KM231765	KM231627	–	–	KM231890	KM232022
Dactylonectria alcacerensis	**CBS 129087**	*V. vinifera*	Portugal	JF735333	KM231629	–	–	JF735819	AM419111
D. estremocensis	**CBS 129085**	*V. vinifera*	Portugal	JF735320	KM231630	–	–	JF735806	JF735448
D. macrodidyma	**CBS 112615**; CPC 3976	*V. vinifera*	South Africa	AY677290	KM515900	–	–	JF735836	AY677233
D. torresensis	**CBS 129086**	*V. vinifera*	Portugal	JF735362	KM231631	–	–	JF735870	JF735492
Ilyonectria capensis	**CBS 132815**; CPC 20695	*Protea* sp.	South Africa	JX231151	KM515908	–	–	JX231119	JX231103
I. destructans	**CBS 264.65**	*Cyclamen persicum*	Sweden	AY677273	KM515927	–	–	JF735695	AY677256
I. leucospermi	**CBS 132809**; CPC 20701	*Leucospermum* sp.	South Africa	JX231161	KM515917	–	–	JX231129	JX231113
I. liriodendri	CBS 117527	*V. vinifera*	Portugal	DQ178165	KM515922	–	–	JF735698	DQ178172
I. mors-panacis	**CBS 306.35**	*Panax quinquefolium*	Canada	JF735288	–	–	–	JF735746	JF735414
I. palmarum	CBS 135753	*Howea forsteriana*	Italy	HF937432	–	–	–	HF922615	HF922609
I. strelitziae	**CBS 142253**; ST6	*Strelitzia reginae*	Italy	*KY304649*	*KY304674*	*KY304621*	*KY304701*	*KY304727*	*KY304755*
	CBS 142254; ST8	*S. reginae*	Italy	*KY304651*	*KY304676*	*KY304623*	*KY304703*	*KY304729*	*KY304757*
	ST7	*S. reginae*	Italy	*KY304650*	*KY304675*	*KY304622*	*KY304702*	*KY304728*	*KY304756*
	ST9	*S. reginae*	Italy	*KY304652*	*KY304677*	*KY304624*	*KY304704*	*KY304730*	*KY304758*
	ST14	*S. reginae*	Italy	*KY304657*	*KY304682*	*KY304629*	–	*KY304735*	*KY304763*
	ST15	*S. reginae*	Italy	*KY304658*	*KY304683*	*KY304630*	*KY304708*	*KY304736*	*KY304764*
	ST25	*S. reginae*	Italy	*KY304668*	*KY304693*	*KY304640*	*KY304718*	*KY304746*	*KY304774*
	ST26	*S. reginae*	Italy	*KY304669*	*KY304694*	*KY304641*	*KY304719*	*KY304747*	*KY304775*
	ST27	*S. reginae*	Italy	*KY304670*	*KY304695*	*KY304642*	*KY304720*	*KY304748*	*KY304776*
	ST28	*S. reginae*	Italy	*KY304671*	*KY304696*	*KY304643*	*KY304721*	*KY304749*	*KY304777*
Macronectria jungneri	CBS 136792	Bark	Brazil	KJ021997	KJ022048	–	–	KJ022375	KJ022313
M. magna	CBS 213.59	*Theobroma cacao*	Guatemala	KJ021981	KJ022034	–	–	KJ022361	KJ022300
M. venezuelana	CBS 136786; G.J.S. 09-1343	Wood	Venezuela	KJ021986	KJ022037	–	–	KJ022365	KJ022303
Neonectria candida	CBS 151.29; IMI 113893	*Malus sylvestris*	England	JF735313	HM042436	–	–	JF735791	JF735438
N. ditissima	CBS 100316	*M. domestica*	Ireland	KM515890	KM515935	–	–	KM515944	DQ789858
N. lugdunensis	CBS 125185; DAOM 235831	*Populus fremontii*	USA	KM231762	KM231625	–	–	KM231887	KM232019

Table 1. (Continued).

Species	Isolate nr.[1]	Substrate	Locality	GenBank Accession no.[2]					
				ITS	LSU	*his3*	*rpb2*	*tef1*	*tub2*
N. neomacrospora	CBS 324.61	*Abies concolor*	The Netherlands	JF735312	HM364318	–	–	HM364335	DQ789875
Pleiocarpon strelitziae	**CBS 142251**; ST1; CPC 27628	*S. reginae*	Italy	*KY304644*	*KY304672*	*KY304616*	*KY304697*	*KY304722*	*KY304750*
	CBS 142252; ST20	*S. reginae*	Italy	*KY304663*	*KY304688*	*KY304635*	*KY304713*	*KY304741*	*KY304769*
	CPC 27629	*S. reginae*	Italy	*KY304645*	–	*KY304617*	*KY304698*	*KY304723*	*KY304751*
	ST3	*S. reginae*	Italy	*KY304646*	–	*KY304618*	–	*KY304724*	*KY304752*
	ST4	*S. reginae*	Italy	*KY304647*	–	*KY304619*	*KY304699*	*KY304725*	*KY304753*
	ST5	*S. reginae*	Italy	*KY304648*	*KY304673*	*KY304620*	*KY304700*	*KY304726*	*KY304754*
	ST10	*S. reginae*	Italy	*KY304653*	*KY304678*	*KY304625*	*KY304705*	*KY304731*	*KY304759*
	ST11	*S. reginae*	Italy	*KY304654*	*KY304679*	*KY304626*	*KY304706*	*KY304732*	*KY304760*
	ST12	*S. reginae*	Italy	*KY304655*	*KY304680*	*KY304627*	*KY304707*	*KY304733*	*KY304761*
	ST13	*S. reginae*	Italy	*KY304656*	*KY304681*	*KY304628*	–	*KY304734*	*KY304762*
	ST17	*S. reginae*	Italy	*KY304660*	*KY304685*	*KY304632*	*KY304710*	*KY304738*	*KY304766*
	ST18	*S. reginae*	Italy	*KY304661*	*KY304686*	*KY304633*	*KY304711*	*KY304739*	*KY304767*
	ST19	*S. reginae*	Italy	*KY304662*	*KY304687*	*KY304634*	*KY304712*	*KY304740*	*KY304768*
	ST21	*S. reginae*	Italy	*KY304664*	*KY304689*	*KY304636*	*KY304714*	*KY304742*	*KY304770*
	ST22	*S. reginae*	Italy	*KY304665*	*KY304690*	*KY304637*	*KY304715*	*KY304743*	*KY304771*
	ST23	*S. reginae*	Italy	*KY304666*	*KY304691*	*KY304638*	*KY304716*	*KY304744*	*KY304772*
	ST24	*S. reginae*	Italy	*KY304667*	*KY304692*	*KY304639*	*KY304717*	*KY304745*	*KY304773*
Rugonectria neobalansae	CBS 125120	Dead tree	Indonesia	KM231750	HM364322	–	–	KM231874	HM352869
R. rugulosa	CBS 126565	Dead tree	Venezuela	KM231749	KM231615	–	–	KM231873	KM232007
Thelonectria discophora	**CBS 134034**; AR 4742	*Tepualia stipularis*	Chile	KC153714	KC121440	–	–	KC153843	KC153779
T. olida	CBS 215.67; ATCC 16548; IMI 116873	*Asparagus officinalis*	Germany	KJ021982	KJ022058	–	–	–	KM232024
	CBS 142255	*S. reginae*	Italy	*KY304659*	*KY304684*	*KY304631*	*KY304709*	*KY304737*	*KY304765*
T. rubi	**CBS 113.12**; IMI 113918	*Rubus idaeus*		KC153718	KC121444	–	–	KC153847	KC153783
T. trachosa	**CBS 112467**; IMI 352560	Bark of conifer	Scotland	AY677297	HM364312	–	–	KM231896	AY677258
T. veuillotiana	CBS 132341; AR 1751	*Eucalyptus* sp.	Azores	JQ403305	JQ403345	–	–	JQ394734	JQ394698
Tumenectria laetidisca	CBS 100284	Bamboo	Japan	KJ022017	KJ022066	–	–	KJ022400	KJ022336
	CBS 101909	Bamboo	Jamaica	KJ022018	KJ022067	–	–	KJ022401	KJ022337
Xenogliocladiopsis cypellocarpa	CBS 133814; CPC 19417	*Eucalyptus cypellocarpa*	Australia	KM231760	KM231623	–	–	KM231885	KM232017

[1]AR: Amy Y. Rossman working collection; ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS;DAOM: Agriculture and Agri-Food Canada National Mycological Herbarium, Canada; G.J.S.: G.J. Samuels working collection; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; PC: P. Chaverri working collection; ST: D. Aiello personal culture numbers. [2]ITS = internal transcribed spacers and intervening 5.8S rDNA, LSU = 28S large subunit ribosomal rDNA, *his3* = histone H3, *rpb2* = RNA polymerase II largest subunit, *tef1* = translation elongation factor 1-alpha, *tub2* = β-tubulin. Epi- and ex-type isolates indicated in **bold**. Sequences generated in this study indicated in *italics*.

Plan-Apochromat × 100/1.4 oil immersion lens (Carl Zeiss, Germany) mounted on a Zeiss Axioscope 2 microscope, with differential interference contrast (DIC) illumination. The 95 % confidence levels were determined for the conidial measurements with extremes given in parentheses. For all other fungal structures measured, only the extremes are provided. Colony colour was assessed using 7-d-old cultures on PDA incubated at room temperature and the colour charts of Rayner (1970). All descriptions, illustrations and nomenclatural data were deposited in MycoBank (Crous *et al.* 2004). Optimal and cardinal growth temperatures were determined by inoculating 90 mm diam PDA plates with a 4 mm diam plug cut from the edge of an actively growing colony. Each isolate was incubated at 4, 10, 15, 20, 25, 30, and 35 °C with three replicate plates per strain at each temperature. Colony diameter of each isolate was determined after 1 wk by measuring the orthogonal directions.

Pathogenicity

Two representative isolates for each phylogenetically resolved species (CBS 142251–142254) were selected for pathogenicity tests on 6-mo-old *Strelizia reginae* plants. Twenty plants were used for each isolate and the same number of plants was used as control. All plants were removed from their original planting substrate, rinsed with water, dipped for 1 min in a 0.3 % (v/v) mixed solution of 5 % (v/v) peracetic acid and 20 % (v/v) hydrogen peroxide (JetFive, Certis Europe), and then rinsed twice with sterile distilled water. Each plant was inoculated with a 4 mm mycelium plug obtained from the margin of an actively growing 14-d-old culture grown on PDA. Each plug was applied to a wound made at the base of the stem using a 4 mm diam cork borer as previously reported (Aiello *et al*. 2014, 2015). Control plants were treated similarly but inoculated with sterile PDA plugs. After inoculation, each plant was wrapped with Parafilm around the inoculation point to prevent desiccation and transplanted into pots containing sterilized growth substrate. All plants were covered with a plastic bag, and maintained in a growth chamber at 25 ºC under a 12 h fluorescent light/dark regimen. All plants were irrigated 2–4 times every week and fertilised every 30 d with 2 g/pot of complex NPK fertilizer Nitrophoska® special (BASF). Plants were evaluated for disease symptoms after 2 and 4 mo.

RESULTS

Field survey and isolation

During the survey, dry basal stem rot symptoms were observed on *Strelizia reginae* in a nursery where these plants are commercially cultivated (Fig. 1). These symptoms were observed on approximately 4 000 of 20 000 potted 2- to 8-yr-old potted plants. Isolations from the symptomatic and bordering healthy tissues consistently yielded cylindrocarpon-like asexual fungi and no *Phytophthora* or any other fungi previously reported from *S. reginae* were isolated.

Phylogenetic analyses

Approximately 325 bases for *tub2*, 500−550 bases for *his3*, ITS and *tef1*, and 850−900 bases for LSU and *rpb2* were determined in this study. Due to the limited sequence data available in GenBank for cylindrocarpon-like fungi for the *his3* and *rpb2* gene regions, both loci could not be included in the phylogenetic inference. The 70 % reciprocal bootstrap tree topologies for the remaining four loci revealed no conflicts and were therefore combined.

The combined alignment of the ITS, LSU, *tef1* and *tub2* contained 2 280 characters from 63 taxa, including *Xenogliocladiopsis cypellocarpa* (CBS 133814; Lombard *et al*. 2015) as outgroup. The number of unique site patterns per data partition, including alignment gaps, was 292 from 570 characters for ITS, 139 from 828 characters for LSU, 377 from 577 characters for *tef1*, and 124 from 305 characters for *tub2*. MrModeltest determined that all four partitions had dirichlet base frequencies. A GTR+I+G model with inverse gamma-distributed rates was used for ITS, LSU and *tef1*, while HKY+I+G with inverse gamma-distributed rates was implemented for *tub2*.

The Bayesian analysis lasted 440 000 generations and the consensus tree, with posterior probabilities, was calculated from 662 trees left after 220 trees were discarded as burn-in. For the MP analysis 1 317 characters were constant, 115 parsimony-uninformative, and 848 parsimony-informative, yielding 216 equally most parsimonious trees (TL = 3566; CI = 0.508; RI = 0.848; RC = 0.431). ML analysis resulted in a single best ML tree with –lnL = -16500.190463. The best ML tree confirmed the consensus tree topologies obtained from the BI and MP analyses, and therefore only the best ML tree is presented (Fig. 2).

In the phylogenetic tree (Fig. 2), the majority of the cylindrocarpon-like isolates obtained from *Strelizia reginae* clustered into two highly-supported clades (both with MP and ML bootstrap support (BS) of 100 % and posterior probabilities (PP) of 1.0) with a single isolate (CBS 142255) clustering (MP-BS & ML-BS = 100 %; PP = 1.0) with the ex-type of *Thelonectria olida* (CBS 215.67). The first group of isolates (including CBS 142253 and CBS 142254) formed a highly-supported clade within the *Ilyonectria* clade, closely related but distinct from the ex-type sequence of *I. palmarum* (CBS 135753), thus representing a novel phylogenetic species within the cylindrocarpon-like genus *Ilyonectria*. The second group of isolates (including CBS 142251 and CBS 142252) formed a highly-supported clade closely related but distinct from the *Thelonectria* clade, representing a previously unrecognized phylogenetically-supported genus.

Pathogenicity

After 10 d, both isolates of the species named below as *Pleiocarpon strelitziae* (CBS 142251 and CBS 142252) induced dry basal stem rot symptoms on *Strelizia reginae* similar to those observed in the nursery (Fig. 3). As a consequence, after 2 mo, all *S. reginae* plants inoculated with *P. strelitziae* died. Both isolates of the species named here as *Ilyonectria strelitziae* (CBS 142254 and CBS 142253; Fig. 3) also induced symptoms of dry basal stem rot as observed in the nursery after 2 mo, but the plants remained alive after 4 mo. All control plants remained healthy throughout the pathogenicity test and none of the test fungi were isolated from them. Re-isolations from the symptomatic plants only yielded the respective inoculated fungi.

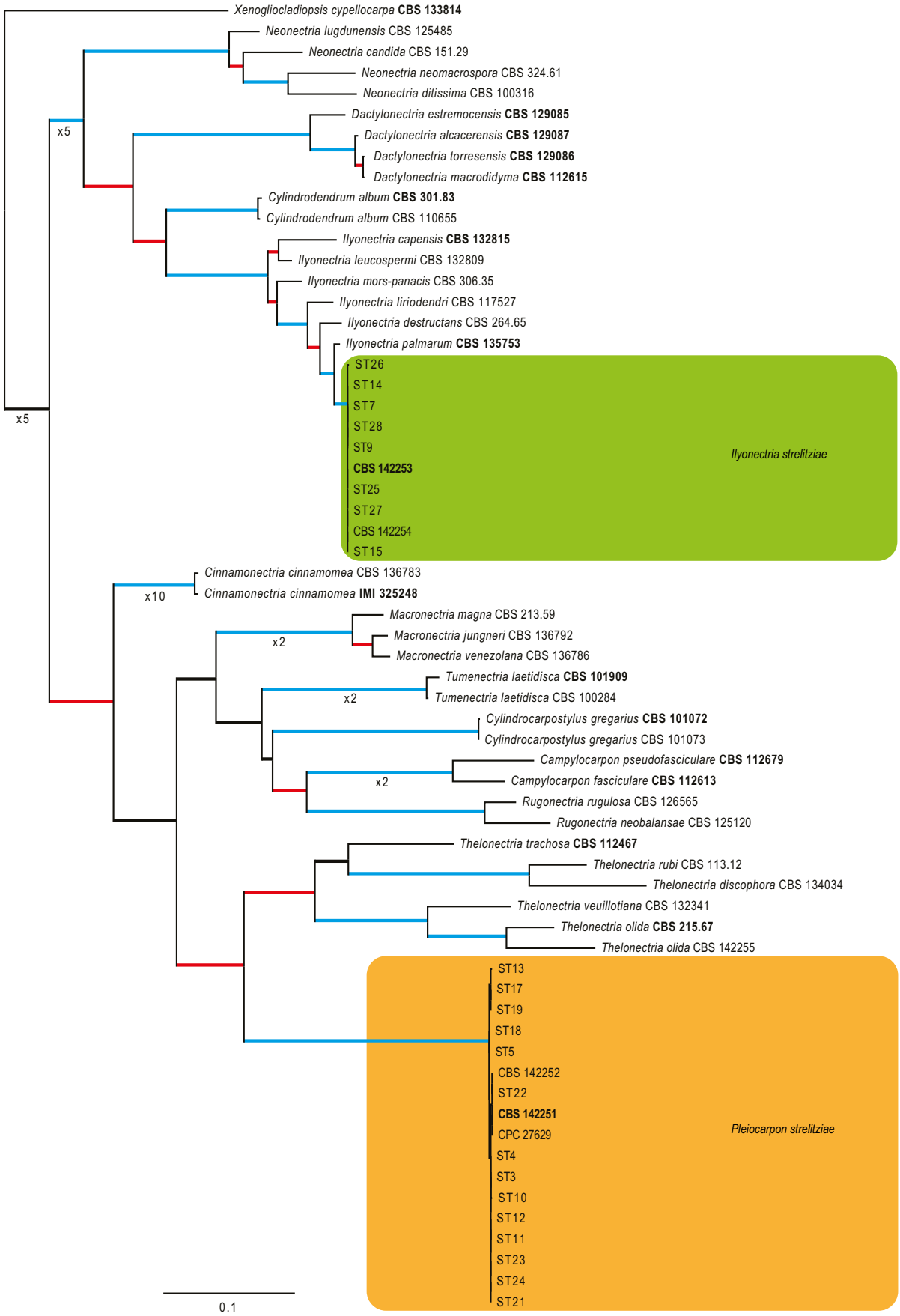

Fig. 2. The ML consensus tree inferred from the combined ITS, LSU, *tef1* and *tub2* sequence alignments. Thickened branches indicate branches present in the ML, MP and Bayesian consensus trees. Branches with ML-BS & MP-BS = 100 % and PP = 1.0 are in blue. Branches with ML-BS & MP-BS ≥ 75 % and PP ≥ 0.95 are in red. The scale bar indicates 0.1 expected changes per site. The tree is rooted to *Xenogliocladiopsis cypellocarpa* (CBS 133814). Epi- and ex-type strains are indicated in bold.

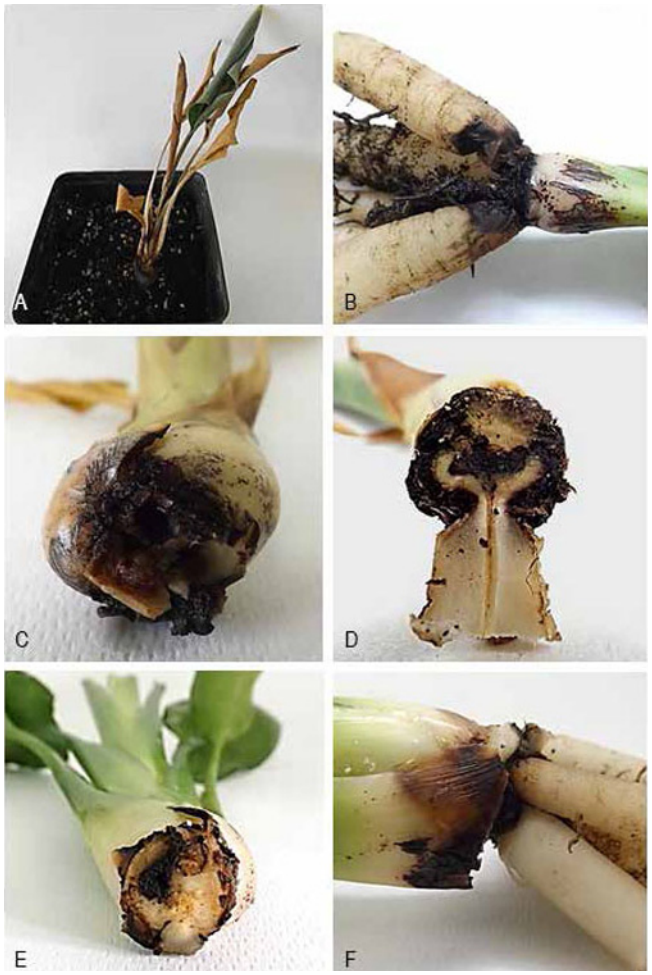

Fig. 3. Symptoms induced during the pathogenicity test on *Strelitzia reginae*. **A–D.** Basal rot and wilting of plant caused by *Pleiocarpon strelitziae*. **E–F.** Basal stem rot and rot of external leaf caused by *Ilyonectria strelitziae*.

TAXONOMY

Morphological observations supported by phylogenetic inference showed that the isolates obtained from the diseased *Strelizia reginae* included a new taxon in the genus *Ilyonectria*, and a novel cylindrocarpon-like genus, both of which are described below.

Ilyonectria strelitziae L. Lombard & D. Aiello, **sp. nov.**
MycoBank MB820027
(Fig. 4)

Etymology: Name derived from the host, *Strelitzia reginae*, from which this fungus was isolated.

Diagnosis: Perithecia orange-red with simple conidiophores arising directly from the surface of the ascomatal wall. Asexual morph producing abundant micro- and macroconidia, but no chlamydospores in culture.

Type: **Italy**: *Sicily*: Catania Province, Carrubba, Riposto, isolated from *Strelitzia reginae*, 2015, *D. Aiello* (CBS H-22967 [dried culture] – holotype; CBS 142253 – ex-type culture).

Description: *Perithecia* formed homothallically *in vitro*, solitary or in groups of 2−3, developing directly on the SNA agar surface, ovoid to obpyriform, orange-red, becoming purple-red in 3 % KOH, finely warted, 275−320 µm diam, to 375 µm high; with simple conidiophores arising directly from the surface of the ascomatal wall, and without a recognisable stroma; perithecial wall consisting of two poorly distinguishable regions; outer region 8−12 µm thick, composed of 2−4 layers of *textura angularis* to *textura globosa*; inner region 3−5 µm thick, composed of 3−4 layers of *textura angularis*. *Asci* subcylindrical to clavate, 25−75 × 4−9 µm, 8-spored; apex truncate to bluntly rounded, with a visible ring. *Ascospores* ellipsoidal, hyaline, tapering towards both ends, divided into two equal sized cells, smooth, (8−)9−11 × 3−4 µm. *Conidiophores* simple, solitary or aggregated into sporodochial-like structures, arising laterally or terminally from aerial mycelium or erect, arising from the agar surface, unbranched or sparsely branched, 1−4-septate, 60−190 µm long, bearing one or rarely two conidiogenous cells. *Conidiogenous cells* monophialidic, cylindrical, tapering slightly towards the apex, 17−60 long, and 2−4 µm wide at the base; micro- and macroconidia produced by simple conidiophores. *Microconidia* abundant, aseptate, ellipsoidal to ovoidal or subcylindrical, straight to slightly curved, with a clearly laterally displaced hilum, 5−7(−9) × 2−3 µm (av. 6 × 2 µm), formed in heads on conidiophores. *Macroconidia* 1−3-septate, straight to slightly curved, base sometimes with a visible, centrally located to laterally displaced hilum; 1-septate macroconidia (9−)11−17(−20) × 2−4 µm (av. 14 × 3 µm); 2-septate macroconidia (14−)18−22(−24) × 3−4 µm (av. 20 × 4 µm); 3-septate macroconidia (19−)21−29(−37) × 3−4 µm (av. 25 × 4 µm). *Chlamydospores* not observed on SNA or PDA.

Culture characteristics: Colonies after 10 d at 24 °C on PDA with cottony, white aerial mycelium in the centre, lacking zonation; centre dark brick becoming cinnamon to honey towards the margins; reverse dark brick in centre, becoming cinnamon towards the margins.

Cardinal growth temperatures: No growth was observed at 4 and 35 °C. Optimal growth was observed at 20 °C, with colonies reaching 265−615 mm diam.

Additional culture examined: **Italy**: *Sicily*: Catania Province, Carrubba, Riposto, isolated from *Strelitzia reginae*, 2015, *D. Aiello* (CBS 142254).

Notes: Based on the phylogenetic inference obtained in this study, *Ilyonectria palmarum* (Aiello *et al*. 2014) is the closest phylogenetic neighbour to *I. strelitziae* (Fig. 2). *Ilyonectria strelitziae* can be distinguished from *I. palmarum* by the simple conidiophores arising from the ascomatal wall, a character not previously reported in *Ilyonectria* (Chaverri *et al*. 2011, Cabral *et al*. 2012a, b, Lombard *et al*. 2013, 2014, 2015). Additionally, *I. strelitziae* readily produced 1-septate macroconidia, not reported for *I. palmarum* (Aiello *et al*. 2014).

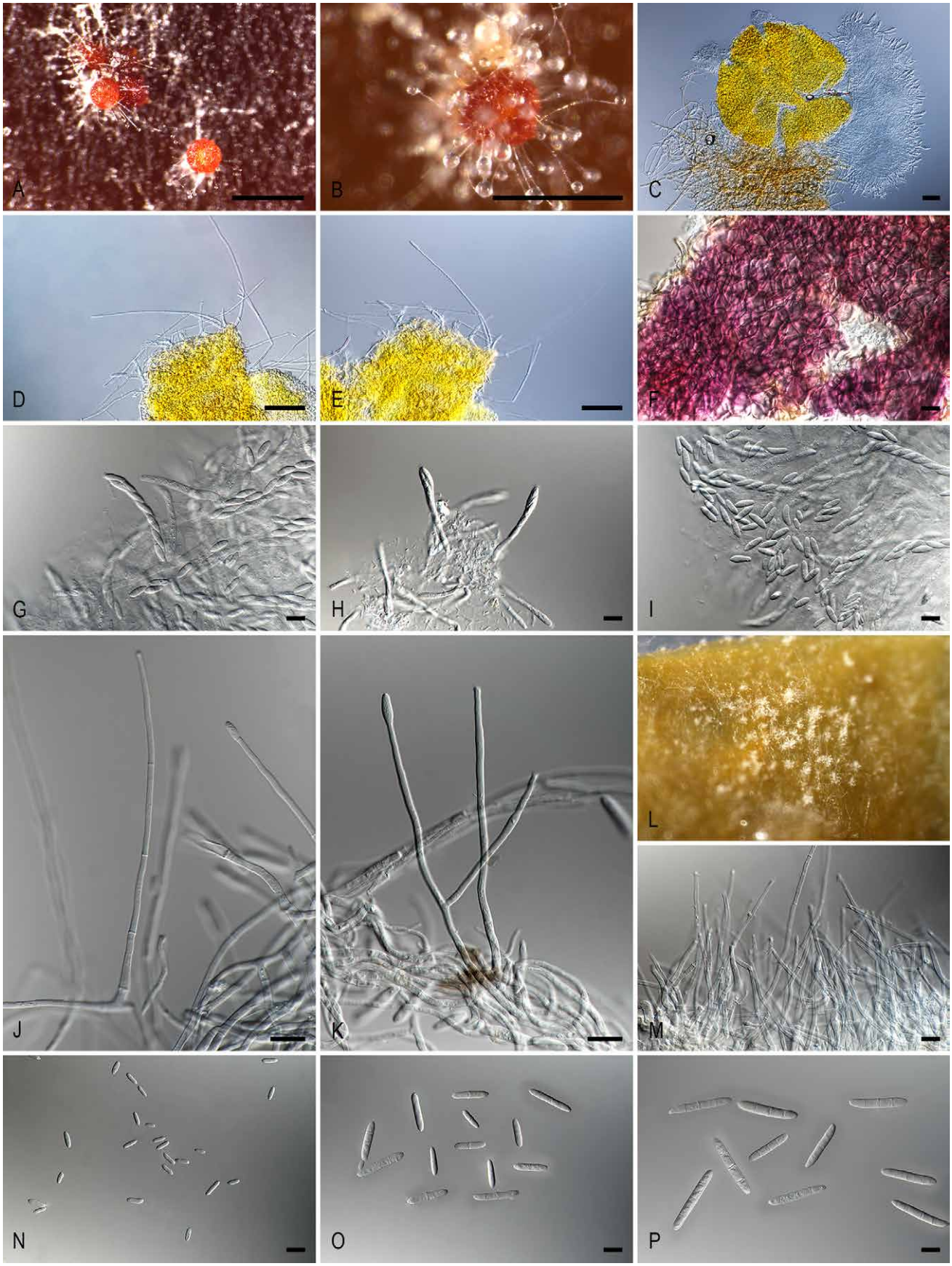

Fig. 4. *Ilyonectria strelitziae* (ex-type culture CBS 142253). **A.** Perithecia on PDA surface. **B.** Perithecium with simple conidiophores arising from the ascomatal wall. **C.** Perithecium exuding asci and ascospores. **D–E.** Simple conidiophores arising from the ascomatal wall. **F.** Ascomatal wall colour reaction in KOH. **G–H.** Asci. **I.** Ascospores. **J–K.** Simple conidiophores on aerial mycelium. **L.** Aggregation of simple conidiophores on carnation leaf. **M.** Aggregation of simple conidiophores. **N.** Microconidia. **O.** 1- and 2-septate macroconidia. **P.** 3-septate macroconidia. Bars A–B = 500 µm; C = 100 µm; D–E = 50 µm; F–K and M–P = 10 µm.

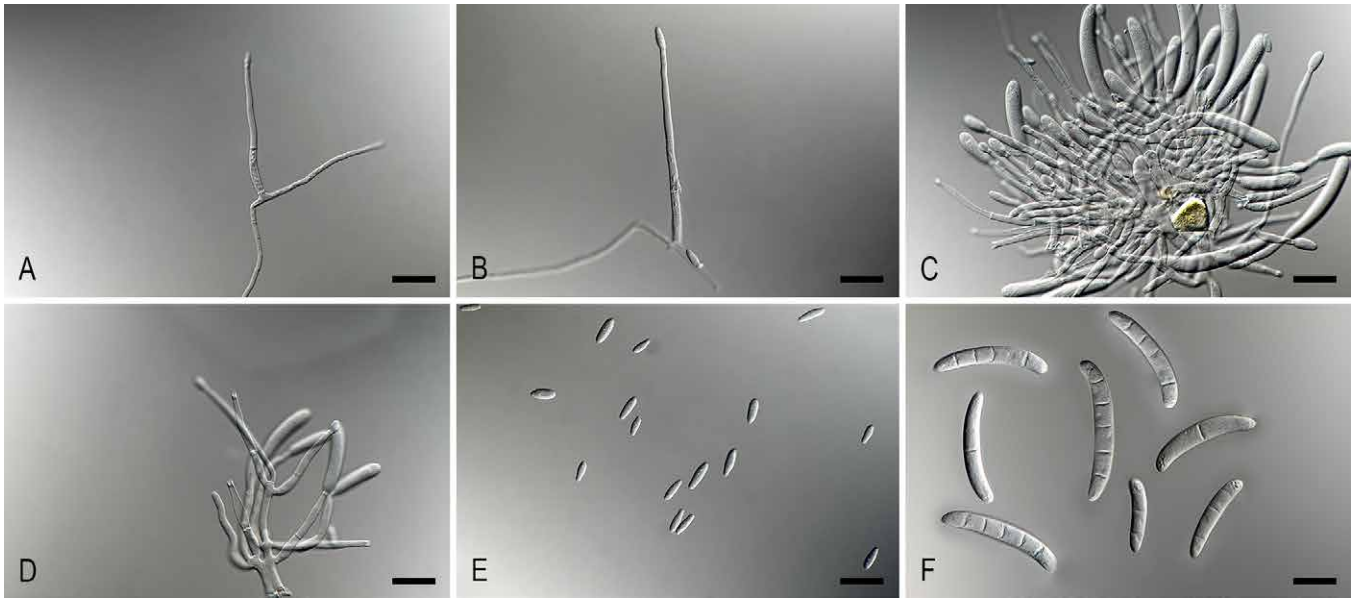

Fig. 5. *Pleiocarpon strelitziae* (ex-type CBS 142251). **A–B.** Simple conidiophores. **C-D.** Sporodochia. **E.** Microconidia. **F.** Macroconidia. Bars = 10 µm

Pleiocarpon L. Lombard & D. Aiello, **gen. nov.**
MycoBank MB820028
(Fig. 5)

Etymology: Named after the highly variable conidial shapes this fungus produces in culture.

Diagnosis: *Sexual morph* unknown. *Asexual morph* cylindrocarpon-like; *microconidia* abundant, aseptate, ellipsoid to ovoid or subcylindrical, straight to slightly curved, with clearly laterally displaced hilum; *macroconidia* cylindrical to subcylindrical, straight to curved, 1−5-septate.

Type species: *Pleiocarpon strelitziae* L. Lombard & D. Aiello 2017.

Description: *Ascomata* not observed. *Conidiophores* simple or aggregated to form sporodochia; simple conidiophores arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, septate, bearing up to two conidiogenous cells. *Conidiogenous cells* monophialidic, cylindrical, tapering slightly towards the apex. *Microconidia* abundant, aseptate, hyaline, ellipsoid to ovoid or subcylindrical, straight to slightly curved, with clearly laterally displaced hilum. *Macroconidia* cylindrical to subcylindrical, hyaline, straight to curved, 1−5-septate, apex or apical cell typically slightly bent to one side and minutely beaked, base with sometimes visible, centrally located or laterally displaced hilum. *Chlamydospores* not observed.

Notes: *Pleiocarpon* is a new cylindrocarpon-like monotypic genus, phylogenetically closely related to the genus *Thelonectria* (Chaverri*et al*. 2011). The asexual morph of *Thelonectria* rarely produces microconidia (Chaverri *et al*. 2012, Salgado-Salazar *et al*. 2016) in contrast to *Pleiocarpon*. Additionally, the macroconidia of the asexual morph of *Thelonectria* are large and up to 9-septate (Chaverri *et al*. 2012, Salgado-Salazar *et al*. 2016), whereas those of *Pleiocarpon* are more intermediate in size and up to 5-septate.

Pleiocarpon strelitziae L. Lombard & D. Aiello, **sp. nov.**
MycoBank MB820029
(Fig. 5)

Etymology: Name derived from the host, *Strelitzia reginae*, from which this fungus was isolated.

Type: **Italy**: *Sicily*: Catania Province, Carrubba, Riposto, isolated from *Strelitzia reginae*, 2015, *D. Aiello* (CBS H-22967 [dried culture] – holotype; CBS 142251 – ex-type culture).

Description: *Ascomata* not observed. *Conidiophores* simple or aggregating to form sporodochia. *Simple conidiophores* solitary, arising laterally or terminally from aerial mycelium, to loosely aggregated, unbranched or sparsely branched, 1−3-septate, 50−85 µm long, bearing one, rarely two conidiogenous cells. *Conidiogenous cells* monophialidic, cylindrical, tapering slightly towards the apex, 22−47 µm long, 2−3 µm wide at the base. *Sporodochia* consisting of a pulvinate mass of short conidiophores, the conidiogenous cells monophialidic, cylindrical, tapering towards the apex, 12−23 µm long, and 2−4 µm wide at the base. *Microconidia* aseptate, with a minute or clearly laterally displaced hilum, ellipsoid to ovoid or subcylindrical, straight to slightly curved, (6−)7−9 × 2−3 µm (av. 8 × 3 µm), formed in heads on simple conidiophores or in hyaline, slimy masses on sporodochia. *Macroconidia* formed by both types of conidiophores, cylindrical to subcylindrical, hyaline, straight to curved, 1−5-septate, apex or apical cell typically slightly bent to one side and minutely beaked, base with sometimes visible, centrally located or laterally displaced hilum; 1-septate macroconidia (19−)27−40 × (3−)5−7 µm (av. 35 × 6 µm); 2-septate macroconidia 23−29(−31) × 5 µm (av. 26 × 5 µm);

3-septate macroconidia (28−)30−40(−46) × 5−6 µm (av. 35 × 6 µm); 4-septate macroconidia (36−)37−41 × 6−7 µm (av. 39 × 6 µm); 5-septate macroconidia (41−)42−47(−50) × 5−7 µm (av. 44 × 6 µm). *Chlamydospores* not observed.

Culture characteristics: Colonies after 10 d at 24 °C on PDA with sparse cottony, white aerial mycelium, lacking zonation; surface and reverse cinnamon to honey.

Cardinal growth temperatures: No growth observed at 4 °C, while colonies grew at 10−35 °C, with optimal growth at 25−30 °C with colonies reaching 410−460 mm diam in one week.

Additional culture examined: **Italy**: *Sicily*: Catania Province, Carrubba, Riposto, isolated from *Strelitzia reginae*, 2015, *D. Aiello* (CBS 142252).

DISCUSSION

Basal stem and root rot of *Strelitzia reginae* can be caused by different fungal species, including *Armillaria mellea*, *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani,* and *Sclerotium rolfsii*. In our study, a new dry basal stem rot was detected on potted *S. reginae* plants cultivated in a nursery in eastern Sicily. Three different cylindrocarpon-like species were consistently isolated from diseased plants, while the above-mentioned pathogens were not found associated with the symptomatic tissues.

According to phylogenetic inference supported by morphological characters, some of the isolates associated with this new dry basal rot of *S. reginae* were identified as a new species of *Ilyonectria*, described here as *I. strelitziae*. Species of this genus are commonly found in soil and cause collar and root diseases on a wide range of plant hosts worldwide (Sánchez *et al*. 2002, Seifert *et al*. 2003, Halleen *et al*. 2004, 2006, Alaniz *et al*. 2007, 2009, Agustí-Brisach *et al*. 2011, Dart & Weeda 2011, Petit *et al*. 2011, Cabral *et al*. 2012a, b, Özben *et al*. 2012, Úrbez-Torres *et al*. 2012, Erper *et al*. 2013, Lombard *et al*. 2013, 2014). In Italy, several species have been reported from ornamental nurseries in eastern Sicily. *Dactylonectria pauciseptata*, *I. novozelandica,* and *I. torresensis,* were reported on potted *Laurus tinus* plants associated with root and crown rot symptoms (Aiello *et al*. 2015); *I. palmarum* associated with dry basal rot of *Arecaceae* (Aiello *et al*. 2014); and *I. macrodidyma* associated with root rot of avocado (*Persea americana*; Vitale *et al*. 2012).

The pathogenicity test in this study showed that *I. strelitziae* induced similar dry basal rot symptoms on *S. reginae* plants to those observed in the nursery. However, nothing is yet known on the origin of this fungal pathogen or its distribution in southern Sicily, which requires further investigation

Some of the remaining isolates obtained from *S. reginae* also had a cylindrocarpon-like morphology, which phylogenetic inference in this study showed to represent a separate genus, here named *Pleiocarpon*, based on the new species *P. strelitziae.* Phylogenetic inference showed that these fungi formed a highly-supported, but distinct, clade closely related to the genus *Thelonectria*. The genus *Thelonectria* was introduced by Chaverri *et al*. (2011) to accommodate fungi with cylindrocarpon-like asexual morphs belonging to Booth's Group 2 (Booth 1966), characterized by lacking microconidia and chlamydospores. Recently, Salgado-Salazar *et al*. (2016) segregated *Thelonectria* by introducing three new genera (*Cinnamomeonectria*, *Macronectria,* and *Tumenectria*) based on phylogenetic inference. *Pleicarpon* can be distinguished from these four genera by the abundant microconidia it produces in culture. Moreover, these genera morphologically resemble *Thelonectria,* and are mostly found on bark or exposed wood of dead, dying or diseased trees, and are rarely associated with small cankers and root rots (Chaverri *et al*. 2011, Salgado-Salazar *et al*. 2016). Pathogenicity tests undertaken in this study demonstrated the aggressive pathogenic nature of *P. strelitziae*, resulting in mortality of all inoculated test plants within 2 mo, which should be of great concern to the cut-flower industry. However, the origin and distribution of this aggressive pathogen still requires further investigation.

One isolate obtained from diseased *S. reginae* plants was identified as *T. olida* based on phylogenetic inference. However, the morphology could not be confirmed, as the isolate did not sporulate on any of the media used. Past phylogenetic studies (Chaverri *et al*. 2011, Salgado-Salazar *et al*. 2016) have shown that this atypical species belongs in the genus *Thelonectria*, although this species produces chlamydospores and has shorter macroconidia. *Thelonectria olida* has been isolated from rotting roots of several plant hosts, but its pathogenicity has never been confirmed (Salgado-Salazar *et al*. 2016). The *T. olida* isolate obtained was excluded from the pathogenicity test undertaken in this study, as its identity was only determined after these had been carried out.

On the basis of the disease incidence and severity observed in the ornamental nursery, we believe that this disease represents a serious threat to potted field-grown plants of *S. reginae*. The cultivation method of pot production could play an important role in promoting infections, since the plants are stressed by frequently being replanting into pots, and wounds can be incurred during transplanting. Moreover, the use of infected soil could represent a further inoculum source for these fungi (Aiello *et al.* 2014).

A wilt disease associated with root rot of *S. reginae* caused by *Cylindrocarpon destructans* was reported in Sicily, but that fungus was identified based only on morphological features (Grasso & Cutuli 1972) and requires critical confirmation.

To our knowledge, this is the first report worldwide of a dry basal stem rot of *S. reginae*, which in Italy was shown to be caused by two new species, *Pleiocarpon strelitziae* and *Ilyonectria strelitziae*.

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.

Aiello D, Guarnaccia V, Epifani F, Perrone G, Polizzi G (2015) '*Cylindrocarpon*' and *Ilyonectria* species causing root and crown

rot disease of potted *Laurustinus* plants in Italy. *Journal of Phytopathology* **163**: 675−680.

Aiello D, Guarnaccia V, Vitale A, Cirvilleri G, Granata G, *et al*. (2014) *Ilyonectria palmarum* sp. nov. causing dry basal stem rot of *Arecaceae*. *European Journal of Plant Pathology* **138**: 347–359.

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

Alaniz S, León M, Vincent A, García-Jiménez J, Abad-Campos P, *et al*. (2007) Characterization of *Cylindrocarpon* species associated with black foot disease of grapevine in Spain. *Plant Disease* **91**: 1187–1193.

Alfieri SA Jr, Langdon KR, Wehlburg C, Kimbrough JW (1984) Index of Plant Diseases in Florida (Revised). *Florida Department of Agriculture and Consumer Services, Division of Plant Industry Bulletin* **11**: 1–389.

Booth C (1966). The genus *Cylindrocarpon*. *Mycological Papers* **104**: 1–54.

Cabral A, Groenewald JZ, Rego C, Oliveira H, Crous PW (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 JZ, *et al*. (2012b) Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. *Fungal Biology* **116**: 62–80.

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

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19−22.

Dart NL, Weeda SM (2011) First report of *Cylindrocarpon macrodidymum* on *Acer palmatum* in Virginia. *Plant Disease* **95**: 1592.

Davino M (1984) Infezioni di *Armillaria mellea* su piante di Strelitzia. *Colture protette* **13**(4): 63−65.

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.

Frisullo F, Babelegoto N, Varvaro L (1987) Danni da *Phytophtora nicotianae* su *Strelitzia reginae* in Puglia. *La difesa delle Piante* **10**: 281–288.

Grasso S, Cutuli G (1972) Un deperimento della "*Strelitzia reginae*" da *Cylindrocarpon destructans Rivista di Patologia Vegetale,* ser. IV **8**: 207–214.

Halleen F, Schroers HJ, 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, Schroers HJ, Groenewald JZ, Rego C, Oliveira H, *et al*. (2006) *Neonectria liriodendri* sp. nov., the main causal agent of black foot disease of grapevines. *Studies in Mycology* **55**: 227−234.

Hilal AA, Helmy AA (1998) Diseases of bird of paradise (*Strelitizia reginae* Banks) in Egypt root and flower rots. *Egyptian Journal of Agricultural Research* **76**: 33−49.

Karsten J (2009) *Envolvimento da peroxidase e polifenoloxidases no bloqueio xilemático de hastes de ave-do-paraiso (Strelitzia reginae)*. Dissertaçãoa, universidade Federal de Viçosa (Mestrado em Fisiologia Vegetal), Viçosa, Minas Gerais, Brasil.

Katho K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772−780.

Kumar S, Stecher G, Tamura K (2015) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870−1874.

Lombard L, Bezuidenhout CM, Crous PW (2013) Ilyonectria black foot rot associated with *Proteaceae*. *Australasian Plant Pathology* **42**: 337−349.

Lombard L, Van der Merwe NA, Groenewald JZ, Crous PW (2014) Lineages in *Nectriaceae*: re-evaluating the generic status of *Ilyonectria* and allied genera. *Phytopathologia Mediterranea* **53**: 515−532.

Lombard L, Van der Merwe NA, Groenewald JZ, Crous PW (2015) Generic concepts in *Nectriaceae*. *Studies in Mycology* **80**: 189−245.

Luongo L, Galli M, Riccioni L, Belisario A (2010) First Report of Leaf Blight and Root and Foot Rot of a *Strelitzia* Caused by *Phytophthora nicotianae* in Italy. *Plant Disease* **94**: 134.

Mason-Gamer R, Kellogg E (1996) Testing for phylogenetic conflict among molecular datasets in the tribe *Triticeae* (*Graminae*). *Systematic Biology* **45**: 524−545.

Nirenberg HI (1981) A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany* **59**: 1599−1609.

Nylander JAA (2004) *MrModeltest* v. 2. Programme distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

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 WF, Rolshausen PE (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, Aiello D, Castello I, Vitale A, Parlavecchio G (2007) First report of southern blight on firewheel tree, bay laurel, bird of paradise, Mediterranean fan palm, and liverwort caused by *Sclerotium rolfsii* in Italy. *Plant Disease* **91**: 1199.

Raabe RD, Connors IL, Martinez AP (1981) *Checklist of Plant Diseases in Hawaii.* Honolulu: Hawaii Institute of Agriculture and Human Resources, College of Tropical Agriculture and Human Resources, University of Hawaii.

Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.

Rego C, Nascimento T, Oliveira H (2001) Characterization of *Cylindrocarpon destructans* isolates from grapevine in Portugal. *Phytopathologia Mediterranea* **40**: S343–S350.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572−1574.

Salgado-Salazar C, Rossman AY, Chaverri P (2013) Not as ubiquitous as we thought: taxonomic crypsis, hidden diversity and cryptic speciation in the cosmopolitan fungus *Thelonectria discophora* (*Nectriaceae*, *Hypocreales*, *Ascomycota*). *PLoS One* **8**(10): e76737.

Salgado-Salazar C, Rossman AY, Chaverri P (2016) The genus *Thelonectria* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and

closely related species with cylindrocarpon-like asexual morphs. *Fungal Diversity* **80**: 411–455.

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

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.

Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312−1313.

Swofford DL (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods).* Version 4.0b10. Sunderland, MA: Sinauer Associates,

Úrbez-Torres JR, Peduto F, Gubler WD (2012) First report of *Ilyonectria macrodidyma* causing root rot of olive trees (*Olea europaea*) in California. *Plant Disease* **96**: 1378.

Van Jaarsveld E (2008) *Strelitzia reginae* Banks ex Aiton. subsp. *Mzimvubuensis* Van Jaarsv. http://www.plantzafrica.com/plantqrs/strelitzregmzimvu.htm.

Vitale A, Aiello D, Guarnaccia V, Perrone G, Stea G, *et al*. (2012) First outbreak of root rot caused by *Neonectria* (*Ilyonectria*) *macrodidyma* on avocado (*Persea americana*). *European Journal of Phytopathology* **160**: 156−159.

Xaba P (2011) *Strelitzia reginae* Aiton. http://www.plantzafrica.com/plantqrs/strelitziareginae.htm.