

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 inflorescences, 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 *Strelitzia 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 (Jeffer

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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 °C) 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- α (*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 (Kato & 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 accelerated [*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-cm² 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 $\times 1000$ magnification using a

Table 1. Strains included in the phylogenetic analyses.

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

Table 1. (Continued).

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

¹AR: 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, CAB International, Egham, Basingstoke, UK; PC: P. Chaverri working collection; ST: D. Aiello personal culture numbers. ²ITS = 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- α , 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 *Strelitzia 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 °C 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 *Strelitzia 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 *Xenoglocladiopsis 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 $-\ln L = -16500.190463$. 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 *Strelitzia 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 *Strelitzia 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.

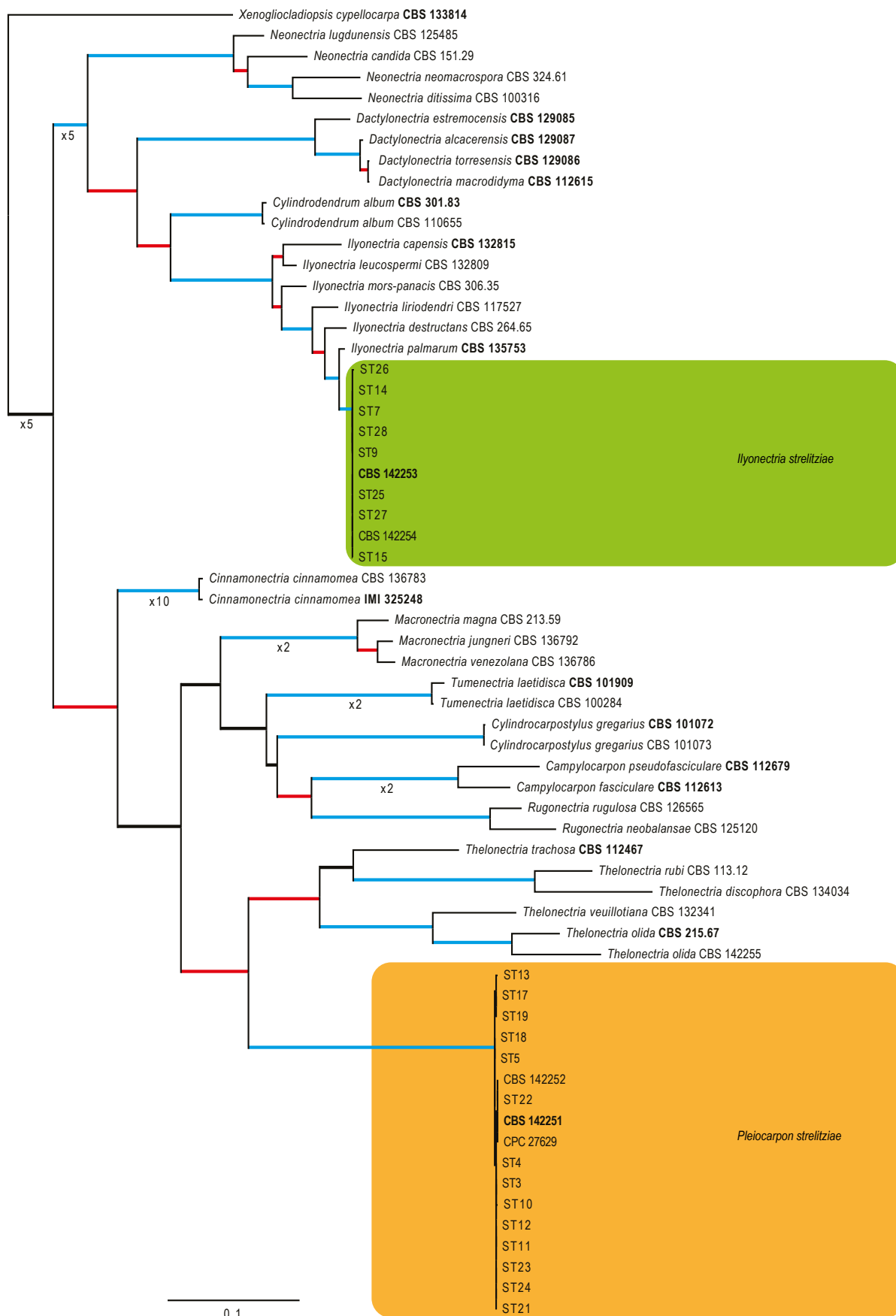


Fig. 2. The ML consensus tree inferred from the combined ITS, LSU, *tef1* and *tub2* sequence alignments. Thickened branches indicate branches present in the ML, MP and Bayesian consensus trees. Branches with ML-BS & MP-BS = 100 % and PP = 1.0 are in blue. Branches with ML-BS & MP-BS ≥ 75 % and PP ≥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

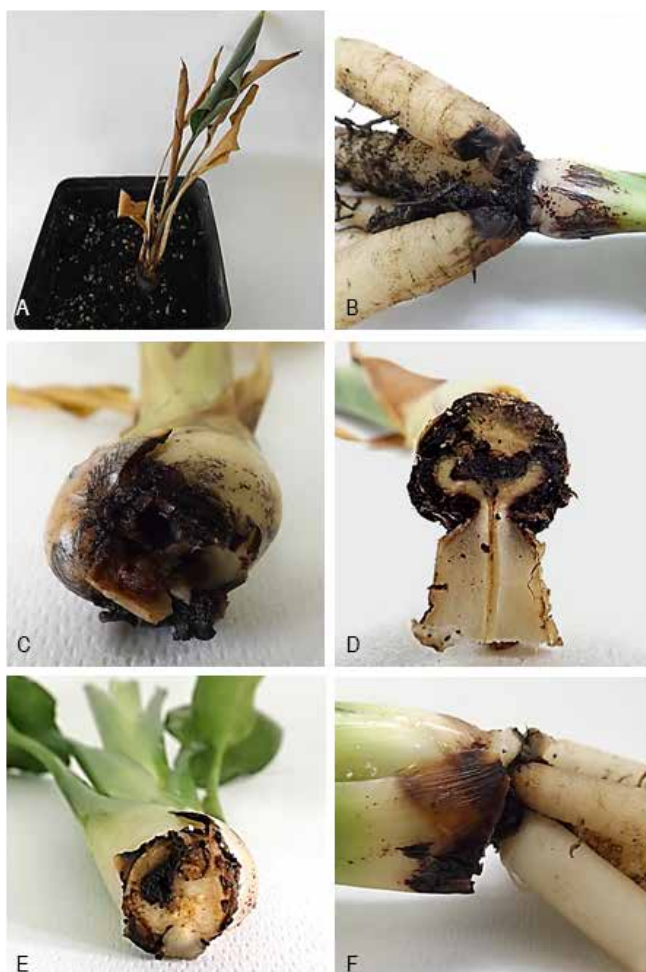


Fig. 3. Symptoms induced during the pathogenicity test on *Strelitzia reginae*. **A–D.** Basal rot and wilting of plant caused by *Pleiocarpon strelitziae*. **E–F.** Basal stem rot and rot of external leaf caused by *Ilyonectria strelitziae*.

TAXONOMY

Morphological observations supported by phylogenetic inference showed that the isolates obtained from the diseased *Strelitzia 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).

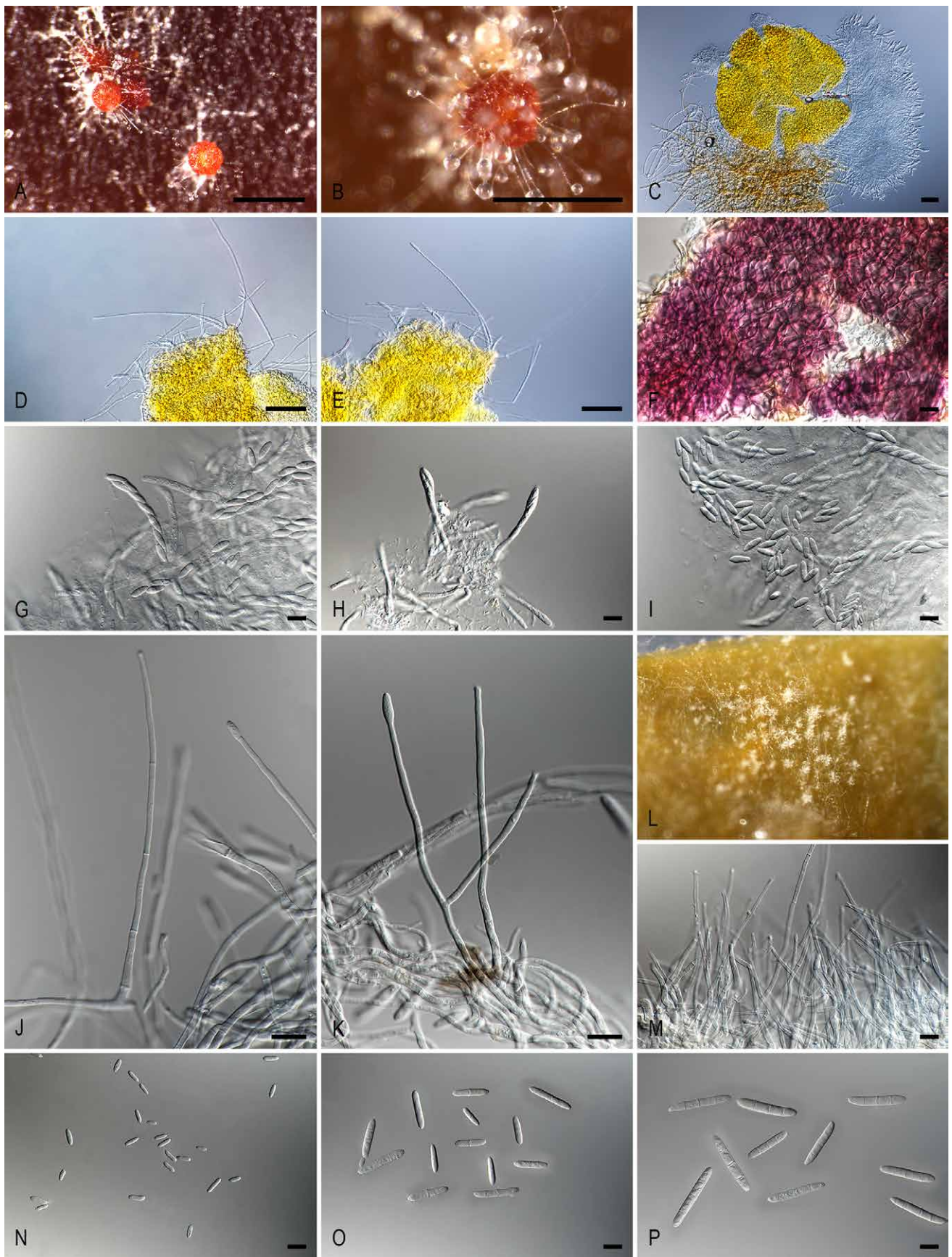


Fig. 4. *Ilyonectria strelitziae* (ex-type culture CBS 142253). **A.** Perithecia on PDA surface. **B.** Perithecium with simple conidiophores arising from the ascomatal wall. **C.** Perithecium exuding asci and ascospores. **D–E.** Simple conidiophores arising from the ascomatal wall. **F.** Ascomatal 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 μ m; C = 100 μ m; D–E = 50 μ m; F–K and M–P = 10 μ m.

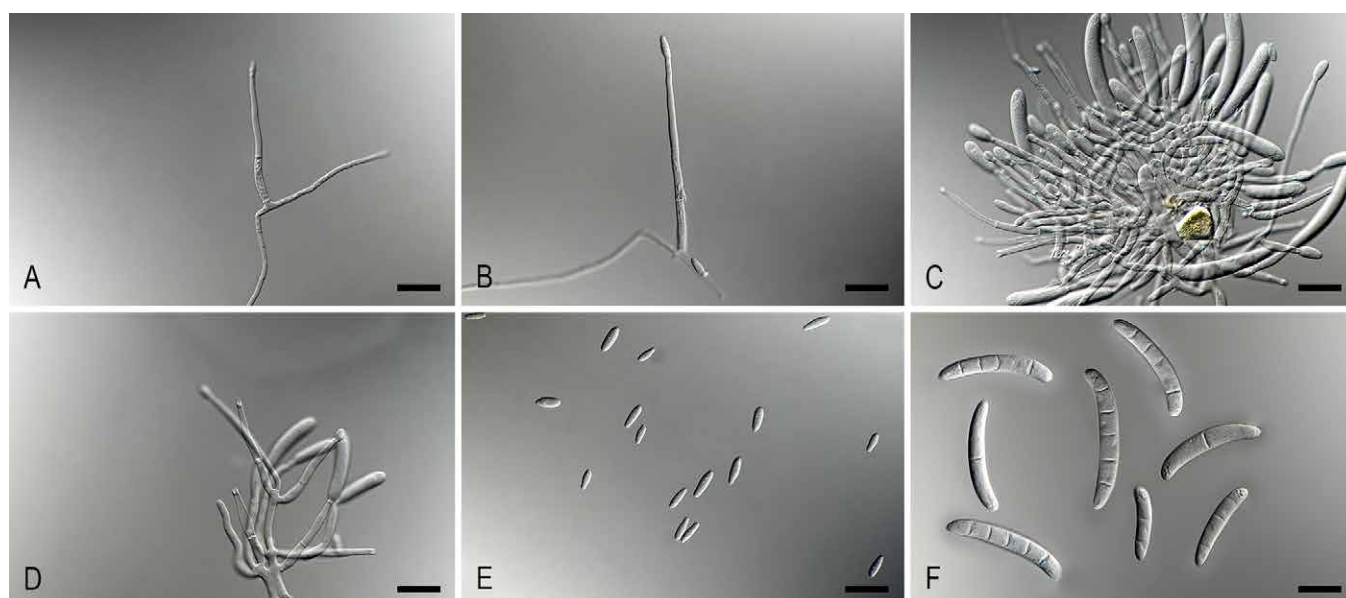


Fig. 5. *Pleiocarpon strelitziae* (ex-type CBS 142251). **A–B.** Simple conidiophores. **C–D.** Sporodochia. **E.** Microconidia. **F.** Macroconidia. Bars = 10 µm

Pleiocarpon L. Lombard & D. Aiello, **gen. nov.**

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(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. *Chlamydoconidia* 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). *Chlamydo*spores 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 chlamydoconidia. Recently, Salgado-Salazar et al. (2016) segregated *Thelonectria* by introducing three new genera (*Cinnamomeonectria*, *Macronectria*, and *Tumenectria*) based on phylogenetic inference. *Pleiocarpon* 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 chlamydoconidia 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*.

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