Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

New or Unusual Disease Reports

A new leaf spot disease of *Chamaerops humilis* caused by *Palmeiromyces chamaeropicola* gen. et sp. nov.

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Summary. In September 2018, a leaf spot disease was noticed on a European fan palm (*Chamaerops humilis* L.) in Oeiras, Portugal. The aim of this study was to identify and characterize the causative agent of this disease symptom. Morphological characters and phylogenetic data derived from ITS and LSU sequences revealed that the leaf spot was caused by a filamentous fungus in the *Mycosphaerellales*, as a unique lineage within the *Teratosphaeriaceae*. This pathogen is introduced here as a new genus and species, *Palmeiromyces chamaeropicola* D.S. Pereira & A.J.L. Phillips, the cause of a newly reported leaf disease on *Chamaerops humilis*.

Keywords. Fungus, new genus, new species, palm tree, plant pathogen.

OPEN ACCESS

Citation: D.S. Pereira, A.J.L. Phillips (2020) A new leaf spot disease of *Chamaerops humilis* caused by *Palmeiromyces chamaeropicola* gen. et sp. nov. *Phytopathologia Mediterranea* 59(2): 353-363. DOI: 10.14601/Phyto-11213

Accepted: June 17, 2020

Published: August 31, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Lizel Mostert, Faculty of AgriSciences, Stellenbosch, South Africa.

INTRODUCTION

Chamaerops humilis L. (*Arecaceae*), commonly known as the European fan palm or Mediterranean dwarf palm, is one of only two indigenous European palms, and the only one found in the Iberian Peninsula (Guzmán *et al*., 2017). This palm tree is distributed around the western Mediterranean Basin, occurring in Europe (Portugal, Spain, France and Italy), North Africa (Morocco, Algeria and Tunisia) and in Mediterranean islands (Balearic, Sicily, Sardinia and Malta) (Dransfield *et al*., 2008; Quattrocchi, 2017; Palmweb, 2020). These trees are used as sources of several products with commercial value, including textiles, food and seeds; they are also important ornamental trees (Guzmán *et al*., 2017). In Portugal, although these palms occur naturally, almost exclusively in the Algarve region (Carapeto *et al*., 2020), they are widely planted and used in gardening and landscaping due to their hardiness and aesthetic value.

Chamaerops humilis is generally free of diseases. No major diseases or pests have been reported for this palm, although it is a potential host for the red palm weevil and other harmful insects (Elliott, 2004). Some reports have shown that the principal fungal diseases on *C. humilis* are leaf spots. For example, this palm is a host for *Graphiola phoenicis*, a leaf spotting

Basidiomycete found exclusively on palms (Piepenbring *et al*., 2012). Fröhlich and Hyde (1998) listed six species of *Mycosphaerella* from leaf spots on different palms, including *M. chamaeropis* on *C. humilis*. More recently, Fusarium wilt and dieback were reported from young and adult *C. humilis* in Spain (Armengol *et al*., 2005), and *Pestalotiopsis chamaeropis* was described from leaves of this host in Italy (Maharachchikumbura *et al*., 2014).

In the present study, a leaf spot disease was noticed on a *C. humilis* palm growing as an ornamental. On the lesions, ascomata, asci and ascospores with characteristics of the *Mycosphaerellales* (Abdollahzadeh *et al*., 2019) were found. Therefore, the purpose of this paper was to characterize the fungus in terms of its morphology and phylogeny.

MATERIALS AND METHODS

Specimen collection and examination

Diseased leaf segments with leaf spots were collected from an ornamental *C. humilis* palm in Oeiras, near Lisbon, Portugal. Plant material was transported to a laboratory in paper envelopes, and examined with a Leica MZ9.5 stereo microscope (Leica Microsystems GmbH) for observations of lesion morphology and associated fungi.

Fungal isolation

A small piece of a leaf spot lesion bearing ascomata was placed on a drop of sterile water in the inverted lid of a Petri dish. The dish base, containing half-strength potato dextrose agar (1/2 PDA) (BIOKAR Diagnostics) was placed on top of the inverted lid. Ascospores were discharged upwards and impinged on the agar surface. After incubating overnight, single germinating ascospores were transferred to fresh plates of 1/2 PDA. Cultures were then incubated in ambient light at room temperature (18–20°C).

Isolations were also made directly from leaf spots. Pieces of leaf spot tissue 1–2 mm^2 were cut from the edge of each lesion, surface sterilized in 5% sodium hypochlorite for 1 min, rinsed in three times in sterile water, and then blotted dry on sterile filter paper. The fragments were plated onto 1/2 PDA containing 0.05% chloramphenicol, and incubated at room temperature until colonies developed.

Morphological observations and characterization

Microscopic structures of isolated fungi were mounted in 100% lactic acid and examined with differential interference contrast (DIC) microscopy. Observations of micromorphological features were made with Leica MZ9.5 and Leica DMR microscopes (Leica Microsystems GmbH), and digital images were recorded, respectively on the two microscopes, with Leica DFC300 and Leica DFC320 cameras (Leica Microsystems GmbH). Measurements were made with the measurement module of the Leica IM500 Image Management System (Leica Microsystems GmbH). Mean, standard deviation (SD) and 95% confidence intervals were calculated from n = total of measured structures. Measurements are presented as minimum and maximum dimensions with mean and SD in parentheses. Photographs were prepared with Adobe Photoshop CS6 (Adobe).

DNA extraction, PCR amplification and sequencing

Genomic DNA (gDNA) was extracted from mycelium of cultures grown on 1/2 PDA, following a modified and optimized version of the guanidium thiocyanate method described by Pitcher *et al*. (1989). PCR reactions were carried out with Taq polymerase, nucleotides, primers, PCR-water (ultrapure DNase/RNase-free distilled water) and buffers supplied by Invitrogen. Amplification reactions were performed in a TGradient Thermocycler (Biometra). Amplified PCR products were purified and sequenced by Eurofins (Germany).

The primers ITS5 (White *et al*., 1990) and NL-4 (O'Donnell, 1993) were used to amplify part of the cluster of rRNA genes, including the nuclear 5.8S rRNA gene and its flanking ITS1 and ITS2 regions, along with the first two domains of the large-subunit rRNA gene (ITS-D1/D2 rDNA region). The PCR reaction mixture consisted of 50–100 ng of gDNA, 1× PCR buffer, 50 pmol of each primer, 200 µM of each dNTP, 2 mM $MgCl_2$, 1 U Taq DNA polymerase, and was made up to a total volume of 50 µL with PCR water. The following cycling conditions were used: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 30 s and elongation at 72°C for 1.5 min, and a final elongation step at 72°C for 10 min.

The ITS region was sequenced only in the forward direction using the primer ITS5. The D1/D2 region (LSU) was sequenced only in the forward direction using the primers ITS5 and NL1 (O'Donnell, 1993). Consensus sequences were produced and edited with BioEdit version 7.0.5.3 (Hall, 1999).

Sequence alignment and phylogenetic analyses

ITS and LSU sequences of species in representative genera of *Teratosphaeriaceae* and *Neodevriesiaceae* in *Mycosphaerellales* were retrieved from GenBank by BLAST searches with the sequences generated in this study (Table 1). These were supplemented with taxa listed in recent literature (e.g. Quaedvlieg *et al*., 2014; Isola *et al*., 2016; Wang *et al*., 2017; Delgado *et al*., 2018). *Capnodium coffeae* Pat. was used as the outgroup taxon representative of a species in *Capnodiales*.

Sequences were aligned with ClustalX version 2.1 (Thompson *et al*., 1997) using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1), and multiple alignment parameters (gap opening = 10, gap extension = 0.2, DNA transition weight = 0.5, delay divergent sequences = 25%). Alignments were checked and manual adjustments were made where necessary with BioEdit. Terminal regions with data missing in some of the isolates were excluded from the analysis. The aligned ITS and LSU sequences were concatenated and combined in a single matrix.

Maximum Likelihood (ML) and Maximum Parsimony (MP) were used for phylogenetic inferences of single gene sequence alignments and the concatenated alignments. The individual gene trees were assessed for clade conflicts between the individual phylogenies by visually comparing the trees generated. ML and MP inferences were implemented on the CIPRES Science Gateway portal version 3.3 (Miller *et al*., 2010), using, respectively, RAxML-HPC2 version 8.2.12 (Stamatakis, 2014) and PAUP version 4.0a165 (Swofford, 2002). The resulting trees were visualized with TreeView version 1.6.6 (Page, 1996).

MP analyses were performed using the heuristic search option with 1000 random taxa additions and Tree Bisection and Reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight, and alignment gaps were treated as missing data. Maxtrees was set to 1000, branches of zero length were collapsed, and all multiple, equally parsimonious trees were retained. Clade stability and robustness of the most parsimonious trees were assessed using bootstrap analysis with 1000 pseudoreplicates, each with ten replicates of random stepwise addition of taxa (Felsenstein, 1985; Hillis and Bull, 1993). Descriptive tree statistics for parsimony included tree length (TL), homoplasy index (HI), consistency index (CI), retention index (RI) and rescaled consistency index (RC).

ML analyses were performed using a General Time Reversible (GTR) nucleotide substitution model including a discrete gamma distribution and estimation of proportion of invariable sites (GTR+G+I) to accommodate variable rates across sites. Clade stability and robustness of the branches of the best-scoring ML tree were estimated by conducting rapid bootstrap analyses with iterations halted automatically by RAxML.

RESULTS

Symptoms and isolations

Symptoms of the disease were found on a single *C. humilis* palm. These were leaf spots randomly distributed on segments of several leaves, which were frequently accompanied by yellowing of the leaf tips and generalized blight (Figure 1). The leaf spots were discrete, circular to ellipsoidal, amphigenous, initially yellowish to brown-grey, each with a wide dark-brown border. The leaf spot centre became progressively greyish and brittle. Each spot was surrounded by a conspicuous yellow or brown to red-brown halo. The discrete spots frequently coalesced, giving rise to blotches of grey necrotic plant tissue. In mature or older spots, the leaf epidermis often flaked off exposing dark ascomata immersed in the necrotic tissue. The bitunicate, obovate to pyriform asci contained eight hyaline, 1-septate ascospores (Figure 1). The lack of pseudoparaphyses suggested that the fungus was closely related to genera in *Teratosphaeriaceae* or *Mycosphaerellaceae*.

Ascospores ejected from ascomata germinated slowly on 1/2 PDA. After 6–12 h 1–2 additional septa formed in each ascospore (Figure 1k and l). After 24–48 h, 1–2 swellings developed on the middle cells and the ascospores became markedly curved (Figure 1m). Further septa developed and germ tubes emerged at right angles to the long axis of each ascospore, all from one side of the ascospore (Figure 1n). Ascospores and germ tubes remained hyaline. The fungus grew slowly forming a black, amorphous mass of mycelium on 1/2 PDA that attained a diameter of *ca*. 3 mm after 3 months of incubation (Figure 1o). No signs of sporulation were visible even after extended periods of incubation of up to 3 months. No other fungi were isolated, even from surface sterilized tissues taken from within the leaf lesions and plated on 1/2 PDA.

Phylogenetic analyses

Results of the BLAST search with ITS and LSU of the fungus isolated from *C. humilis* revealed that it was closely related to species in *Teratosphaeriaceae* and *Neodevriesiaceae*, and only distantly related to *Mycosphaerellaceae*. The available ITS and LSU sequences of 81 strains of *Teratosphaeriaceae* and *Neodevriesi-*

Table 1. Isolates used in the phylogenetic analyses of *Palmeiromyces chamaeropicola*.

Taxon	Strain number[a]	Status[b]	GenBank accession number[c]	
			ITS	LSU
Acrodontium pigmentosum	CBS 111111	T	KX287275	KX286963
Batcheloromyces alistairii	CBS 120035	T	DQ885901	KF937220
B. alistairii	CPC 18251		JX556227	JX556237
B. leucadendri	CBS 114146		–	EU707892
B. proteae	CBS 110696		JF746163	KF901833
B. sedgefieldii	CBS 112119	T	EU707893	KF937222
Camarosporula persooniae	CBS 112494		JF770448	JF770460
Capnodium coffeae	CBS 147.52		MH856967	MH868489
Devriesia staurophora	CBS 375.81		KF442532	KF442572
D. thermodurans	CBS 115878	T	MH862991	MH874549
Eupenidiella venezuelensis	CBS 106.75	T	KF901802	KF902163
Hortaea thailandica	CBS 125423	T	MH863702	MH875167
H. werneckii	CBS 107.67	T	AJ238468	EU019270
H. werneckii	CBS 359.66		MH858825	MH870461
Meristemomyces arctostaphylos	CBS 141290	T	KX228264	KX228315
M. frigidus	CBS 136109	T	KF309971	GU250401
Myrtapenidiella corymbia	CBS 124769	T	KF901517	KF901838
M. eucalypti	CBS 123246	T	KF901772	KF902130
M. tenuiramis	CBS 124993	T	KF937262	GQ852626
Neodevriesia agapanthi	CBS 132689	T	KJ564346	JX069859
N. coccolobae	CBS 145064	T	MK047432	MK047483
N. coryneliae	CBS 137999	T	KJ869154	KJ869211
N. hilliana	CBS 123187	T	MH863277	MH874801
N. imbrexigena	CAP 1373	T	JX915746	JX915750
N. imbrexigena	CAP 1375		JX915748	JX915752
N. knoxdaviesii	CBS 122898	T	EU707865	EU707865
N. knoxdaviesii	CPC 14905		EU707866	KJ564328
N. lagerstroemiae	CBS 125422	T	GU214634	KF902149
N. shakazului	CBS 133579	T	KC005776	KC005797
N. stirlingiae	CBS 133581	T	KC005778	KC005799
N. strelitziae	CBS 122379	T	EU436763	GU301810
N. tabebuiae	CBS 145065	T	MK047433	MK047484
N. xanthorrhoeae	CBS 128219	T	HQ599605	HQ599606
Neophaeothecoidea proteae	CBS 114129	T	MH862955	KF937228
Palmeiromyces chamaeropicola	CDP 001	T	MT068628	MT076194
Parapenidiella pseudotasmaniensis	CBS 124991	T	MH863440	MH874943
Para. tasmaniensis	CBS 111687	T	KF901521	KF901843
Penidiella carpentariae	CBS 133586	T	KC005784	KC005806
Pen. columbiana	CBS 486.80	T	KF901630	KF901965
Pseudotaeniolina globosa	CBS 109889	T	KF309976	KF310010
Pseudoteratosphaeria flexuosa	CBS 111012	T	KF901755	KF902110
Pseudo. flexuosa	CBS 111048		KF901643	KF901978
Pseudo. ohnowa	CBS 112896	T	KF901620	KF901946
Queenslandipenidiella kurandae	CBS 121715	T	KF901538	KF901860
Readeriella dendritica	CBS 120032	T	KF901543	KF901865
R. limoniforma	CBS 134745	T	KF901547	KF901869
R. mirabilis	CBS 125000	ET	KF901549	KF901871

(Continued)

Table 1. (Continued).

Taxon	Strain number[a]	Status[b]	GenBank accession number[c]	
			ITS	LSU
Stenella araguata	CBS 105.75	T	MH860897	MH872633
Zasmidium musae	CBS 122477	T	EU514291	–
Z. musae	CBS 121385		EU514293	–
Teratosphaeria alboconidia	CBS 125004	T	KF901558	KF901881
Ter. biformis	CBS 124578	T	KF901564	KF901887
Ter. blakelyi	CBS 120089	T	KF901565	KF901888
Ter. brunneotingens	CPC 13303	T	EF394853	EU019286
Ter. complicata	CBS 125216	T	MH863461	MH874961
Ter. complicata	CPC 14535		KF901781	KF902139
Ter. considenianae	CBS 120087	T	DQ923527	KF937238
Ter. considenianae	CPC 14057		KF901568	KF901892
Ter. cryptica	CBS 110975		KF901573	KF901897
Ter. cryptica	CBS 111679		KF901691	KF902037
Ter. dimorpha	CBS 120085		DQ923529	KF937240
Ter. dimorpha	CBS:124051		KF901575	KF901899
Ter. encephalarti	CBS 123540	T	FJ372395	FJ372412
Ter. encephalarti	CPC 15466		FJ372401	FJ372418
Ter. hortaea	CBS 124156	T	MH863358	MH874881
Ter. hortaea	CPC 15723		FJ790279	FJ790300
Ter. macowanii	CBS 122901	ET	MH863257	MH874781
Ter. macowanii	CPC 1488		AY260096	FJ493199
Ter. mareebensis	CBS 129529	T	JF951149	JF951169
Ter. maxii	CBS 120137	T	DQ885899	KF937243
Ter. maxii	CBS 112496		EU707871	KF937242
Ter. micromaculata	CBS 124582	T	MH863390	MH874909
Ter. profusa	CBS 125007	T	KF901592	KF901916
Ter. profusa	CPC 12821		FJ493196	FJ493220
Ter. rubidae	CBS 124579	T	MH863388	MH874907
Ter. rubidae	MUCC 659		EU300992	–
Ter. sieberi	CBS 144443	T	MH327816	MH327852
Ter. wingfieldii	CBS 112163	T	EU707896	–
Teratosphaericola pseudoafricana	CBS 114782	T	KF901737	KF902084
Terph. pseudoafricana	CBS 111168		KF901699	KF902045
Teratosphaeriopsis pseudoafricana	CBS 111171	T	KF901738	KF902085

[a]CAP = Culture Collection of Alan Phillips, housed at M&B-BioISI, Tec Labs, University of Lisbon, Lisbon, Portugal, CBS = Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, CDP = Culture Collection of Diogo Pereira, housed at M&B-BioISI, Tec Labs, University of Lisbon, Lisbon, Portugal, CPC = Culture Collection of Pedro Crous, housed at the Westerdijk Institute,, MUCC = Murdoch University Culture Collection, Murdoch, Australia.
[b]Status of the strains, T = ex-type, ET = ex-epitype.
[c]Newly generated sequences are in bold font.

aceae, either sequenced in this study or retrieved from GenBank, were included in the phylogenetic analysis (Table 1). The concatenated ITS and LSU alignment of 80 ingroup taxa and one outgroup taxon comprised 1152 characters (including alignment gaps), with 599 characters for ITS and 553 for LSU, after alignment. Tree topologies resulting from maximum parsimony and maximum likelihood analyses were similar, and both presented well-resolved clades for each genus included in the analyses, mostly supported by high bootstrap values (≥ 70%). The ML tree is shown in Figure 2.

Of the 1152 characters, 653 were constant and 139 variable characters were parsimony-uninformative. MP analysis of the remaining 364 parsimony-informative

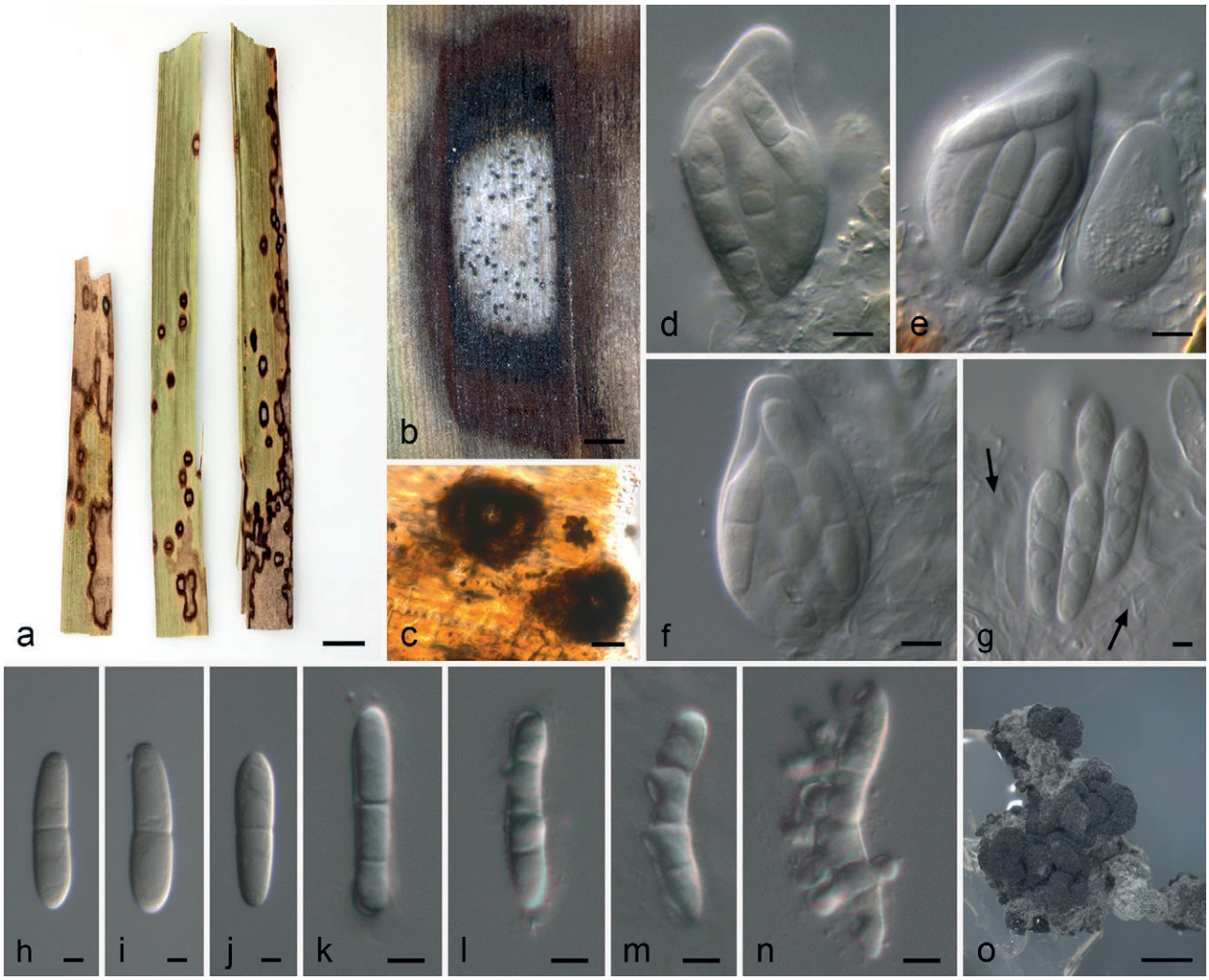

Figure 1. *Palmeiromyces chamaeropicola* (CDP 001, ex-type). a, Leaf spots on segments of *Chamaerops humilis*. b and c, Appearance of ascomata on host leaf surface. d, e and f, Asci and cellular hamathecium remnants. g, Immature ascospores (black arrows indicate remnants of cellular hamathecium). h, i and j, Mature ascospores. k, l, m and n, Germinating ascospores (k, after *ca.* 6 h incubation; l, after *ca.* 12 h incubation; m, after *ca.* 24 h incubation; n, after *ca.* 48 h incubation). o. Three-month-old colony on 1/2 PDA. Scale bars: a = 10 mm, b = 0.5 mm, c = 30 µm, d, e and f = 5 µm, g, h, i and j = 2.5 µm, k, l, m and n = 5 µm, o = 1 mm.

characters resulted in 140 equally parsimonious trees of 2181 steps and a moderately high level of homoplasy as indicated by a CI of 0.381, an RI of 0.693, an HI of 0.619 and an RC of 0.264. Topology of the trees differed from one another only in the position of the isolates within the terminal groupings of the *Teratosphaeria* clade. All other clades were consistent in their phylogenetic positions.

The final likelihood score for the ML tree was -11854.411424. The matrix had 560 distinct alignment patterns, with 15.85% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.222286, C = 0.271515, G = 0.286947 and T = 0.219253; substitution rates AC = 1.523961, AG = 1.983358, AT = 1.573616, CG = 1.073620, CT = 5.812546 and GT = 1.000000; gamma distribution shape parameter α = 0.554479; proportion of invariable sites = 0.384063.

Although well-resolved, internal nodes within *Teratosphaeriaceae* received low bootstrap support (≤ 50%). Nevertheless, *Teratosphaeriaceae* and *Neodevriesiaceae* were well-separated, insomuch that represented a clade with 100% bootstrap support, which confirms the phylogenetic difference that supports these two families.

The isolate obtained from *C. humilis* clustered in a separate and previously undescribed lineage among the selected genera in *Teratosphaeriaceae* and *Neodevriesiaceae*. Nevertheless, its placement between these two

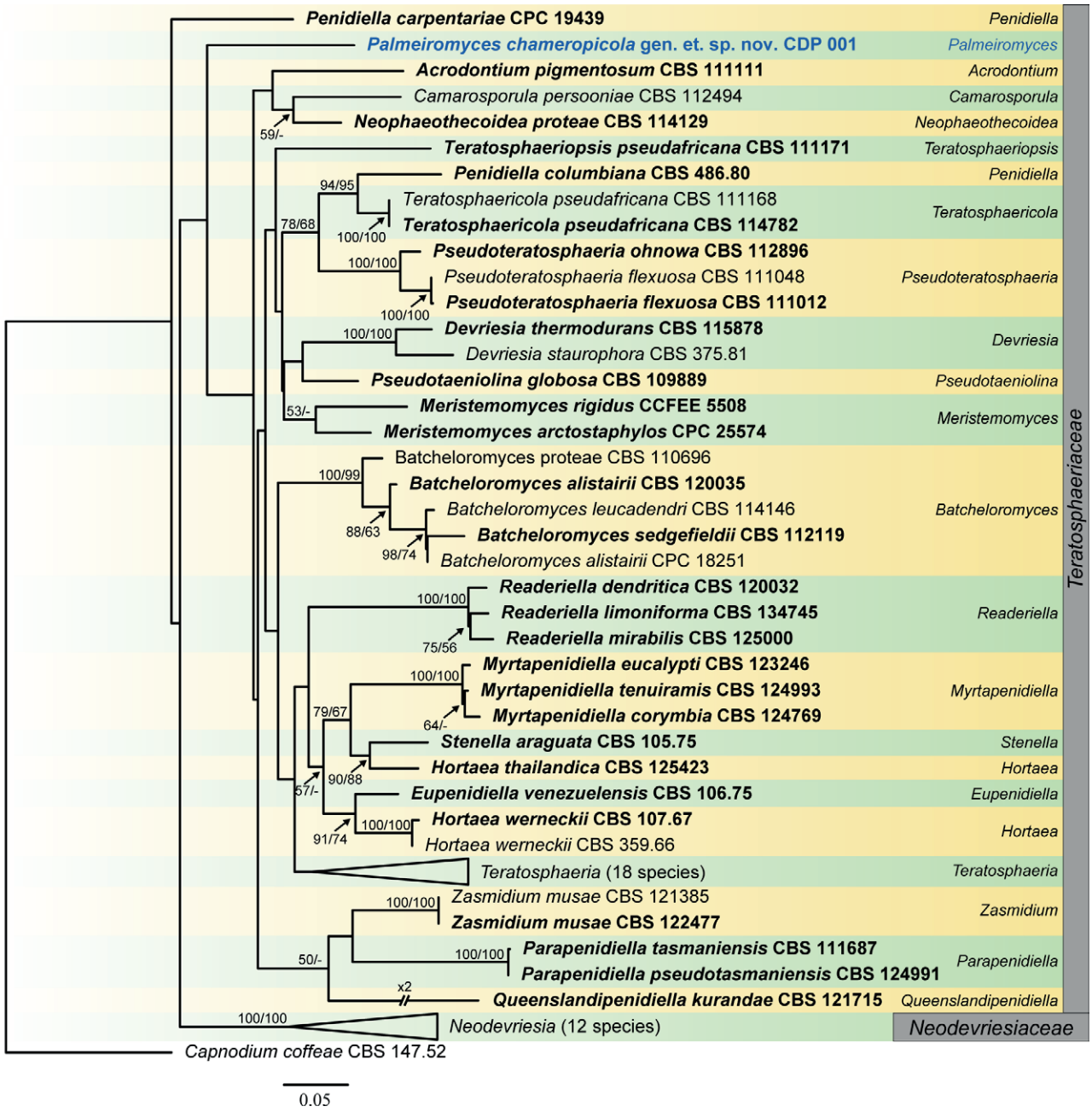

Figure 2. Maximum likelihood tree of *Teratosphaeriaceae* and *Neodevriesiaceae* based on combined ITS and LSU sequences. ML/MP bootstrap support values (> 50%) are shown above the branches. The isolate from *Chamaerops humilis* is indicated in blue font. The scale bar represents the expected number of nucleotide changes per site. Ex-type/ex-epitype isolates are in bold font.

families received low bootstrap support. Considering the results from both MP and ML analyses, this fungus clusters close to genera in *Teratosphaeriaceae*. A total of nine unique base pair differences in the ITS locus and five in the LSU locus among the 81 isolates included in the phylogenies confirms the novel lineage of the isolate as a new genus here introduced in *Mycosphaerellales*.

Taxonomy

Based on DNA phylogeny and morphology, the isolate collected from leaf spots on *C. humilis* was distinct from all other known species and genera in *Mycosphaerellales*. The data presented here indicate that this fungus resides in *Teratosphaeriaceae* as a new spe-

cies and a new genus. Descriptions of the fungus are provided below.

Palmeiromyces D.S. Pereira & A.J.L. Phillips, gen. nov.
MycoBank: MB 834638

Etymology: Named for the Portuguese word for palms (palmeiras), the host on which it was found.

Type species: *Palmeiromyces chamaeropicola* D.S. Pereira & A.J.L. Phillips, sp. nov.

Ascomata pseudothecial, amphigenous, subepidermal, immersed to erumpent, scattered or clustered, globose to subglobose, dark-brown, ostiolate. *Ostiole* circular, aperiphysate. *Peridium* thin-walled, composed of cells forming a *textura angularis*, outer layer composed of thick-walled, dark-brown to brown cells, inner layers composed of thin-walled, hyaline cells. *Pseudoparaphyses* absent. *Asci* bitunicate, fissitunicate, pyriform to obovoid, with well-developed ocular chamber, smooth-walled, hyaline, 8-spored. *Ascospores* biseriate, hyaline, broadly ellipsoidal to subcylindrical, with rounded ends, smooth- and thin-walled, medianly 1-septate, not constricted at the septum.

Palmeiromyces chamaeropicola D.S. Pereira & A.J.L. Phillips, sp. nov. (Figure 1)
MycoBank: MB 834639

Etymology: Named for *Chamaerops humilis*, the host genus from which it was collected.

Holotype: AVE-F-11

Leaf spots sunken, circular to broadly ellipsoidal, 3–7 × 2–3 mm (mean ± SD = 4.67 ± 1.06 × 2.52 ± 0.51 mm), identical on both leaf surfaces, brown-grey to yellowish centre, later becoming greyish and fragile, with a dark-brown border (*ca.* 1 mm wide), surrounded by a conspicuous yellow or brown to red-brown halo, occasionally coalescing, randomly distributed. Mature leaf spots contain several immersed ascomata. *Ascomata* pseudothecial, amphigenous, subepidermal, immersed to erumpent, scattered or clustered in groups of two or three, globose to subglobose, dark-brown, up to 90 µm diam., ostiolate. *Ostiole* circular, up to 21 µm diam., aperiphysate. *Peridium* thin-walled, composed of cells forming a *textura angularis*, outer layer composed of thick-walled, dark-brown to brown cells, inner layers composed of thin-walled, hyaline cells. *Pseudoparaphyses* absent, but pseudoparenchymatous, cellular hamathecium remnants present. *Asci* bitunicate, outer wall up to 2 µm thick, fissitunicate, pyriform to obovoid, slightly curved, broader at the base, well-developed ocular chamber, smooth-walled, hyaline, 8-spored, 21.4–57.9 × 8.2–19.1 µm (mean ± SD = 33.22 ± 9.69 × 14.20 ± 3.75 µm, n = 11). *Ascospores* biseriate, broadly ellipsoidal to subcylindrical, with rounded ends, occasionally slightly curved, hyaline, smooth- and thin-walled, guttulate, medianly 1-septate, not to slightly constricted at the septa, 9.5–21.9 × 2.8–5.5 µm (mean ± SD = 17.31 ± 3.23 × 4.05 ± 0.72 µm, n = 50); mean ± SD ascospore length/width ratio = 4.30 ± 0.58 (n = 50).

Material examined: PORTUGAL, Oeiras, National Sports Centre of Jamor, on leaf spots of *Chamaerops humilis* (*Arecaceae*), 20 September 2018, Alan J.L. Phillips (holotype AVE-F-11, ex-type living culture CDP 001).

GenBank Numbers: ITS: MT068628; LSU: MT076194.

Distribution: Oeiras, Portugal.

Host: *Chamaerops humilis*.

Notes: *Palmeiromyces chamaeropicola* was found associated with leaf spots of *C. humilis*, but pathogenicity has not been tested. Nevertheless, there is evidence that this species represents an obligate biotroph causing a previously undescribed disease on *C. humilis*. Phylogenetically, *P. chamaeropicola* is closely related to genera in *Teratosphaeriaceae* (Figure 2). Morphology of the sexual morph, ascospores with a peculiar mode of germination, lack of an asexual morph and very slow growth in culture correspond to genera in *Teratosphaeriaceae* (Crous *et al*., 2007). However, ascospores of *P. chamaeropicola* lack mucous sheaths, which is a characteristic of *Teratosphaeriaceae* (Crous *et al*., 2007; Quaedvlieg *et al*., 2014).

DISCUSSION

In this study, a new species in *Teratosphaeriaceae*, *Palmeiromyces chamaeropicola*, is described and a new genus is established to accommodate the fungus. Phylogenetic analyses based on ITS and LSU sequences revealed that *Palmeiromyces* represents a separate lineage close to several *Teratosphaeriaceae* genera, as well as to *Neodevriesiaceae*. The evidence gained from unique nucleotide differences among the several genera included in the phylogeny supports this novelty at genus-level. This species was associated with, and is considered to be the cause of, a leaf spot disease of the palm *C. humilis*.

Morphologically *Palmeiromyces chamaeropicola* resembles *Mycosphaerellaceae* and *Teratosphaeriaceae*, characterized by small, inconspicuous ascomata immersed in the host tissue, which produce pyriform asci with eight hyaline, ellipsoidal and medianly 1-septate ascospores. The presence of pseudoparenchymatal remnants in ascomata of *Palmeiromyces* and the absence of paraphyses place it within *Teratosphaeriaceae*, since Crous *et al*. (2007) used these characters to separate

Teratosphaeriaceae from *Mycosphaerellaceae*. Nevertheless, the low bootstrap support for the internal nodes and thus for the branches between *P. chamaeropicola* and the remaining taxa in the phylogenetic analyses suggest that future studies may reveal a different phylogenetic position for this taxon, possibly as a new family. Genera in *Teratosphaeriaceae* and *Mycosphaerellaceae* are often defined based on DNA sequence data, and on morphology of their asexual morphs. However, *P. chamaeropicola* barely grew in culture with no signs of asexual sporulation. This is common in *Teratosphaeria* species, which are cultivated with difficulty (Crous *et al*., 2007, 2009c). Morphologically *P. chamaeropicola* resembles *Mycosphaerella cocoës*, which was found associated with leaf spots on various palm hosts, such as *Calamus*, *Cocos* and *Mauritia* (Fröhlich and Hyde, 1998). However, no cultures linked to the holotype of *M. cocoës* are extant and thus no DNA data are available for this species. Comparison of species within *Mycosphaerellaceae* and *Teratosphaeriaceae* solely on the basis of morphology is unreliable (Hunter *et al*., 2006; Crous *et al*., 2008, 2009c). Crous *et al*. (2008) noted that the morphological species concept had in the past obscured the presence of novel taxa, which have been resolved by means of molecular analyses. Therefore, it was not possible to determine the phylogenetic relationship between *P. chamaeropicola* and *M. cocoës*.

The phylogenetic position of *P. chamaeropicola* within *Mycosphaerellales* is uncertain and no accurate nearest neighbours could be indicated in the present analyses. In addition, a high level of homoplasy was detected in the MP analysis. Most genera within *Teratosphaeria* are polyphyletic, and within *Capnodiales* are paraphyletic (Crous *et al*., 2007). Convergence is observed in several genera, especially with respect to the morphology of asexual morphs (Crous *et al*., 2007, 2009b; Ruibal *et al*., 2008; Quaedvlieg *et al*., 2014). *Capnodiales* was recently sub-divided into seven orders (Abdollahzadeh *et al*., 2019) with *Mycosphaerellaceae* and *Teratosphaeriaceae* accommodated in *Mycosphaerellales*.

The phylogenetic analyses in this study suggest that *Palmeiromyces* could represent a new family, although it is not regarded as such in the present study, mainly due to low taxon sampling for the new genus. Besides the DNA phylogenetic data, *P. chamaeropicola* lacks several morphological characters that are diagnostic for *Teratosphaeria*, the type genus of *Teratosphaeriaceae*. These characters include ascospores that turn brown and verruculose while still in the asci, as well as the presence of mucoid sheaths around the ascospores (Quaedvlieg *et al*., 2014), all of which are lacking in *Palmeiromyces*. In addition, the germination pattern of ascospores of *Palmeiromyces* is completely distinct from those reported in *Mycosphaerellaceae*, although this pattern is seen in some species in *Teratosphaeriaceae*. Thus, *Palmeiromyces* clearly represents a separate genus within *Mycosphaerellales* where several phylogenetic lineages remain poorly resolved due to limited taxon sampling (Quaedvlieg *et al*., 2014). However, its position within *Teratosphaeriaceae* cannot be established and it is possible that future studies with greater taxon sampling may split *Palmeiromyces* from *Teratosphaeriaceae*.

Palmeiromyces chamaeropicola was collected from diseased foliage of *C. humilis* and reveals a new insight into the *Teratosphaeriaceae* leaf diseases (TLD) and *Mycosphaerellaceae* leaf diseases (MLD). Although the pathogenicity of *P. chamaeropicola* has not been tested, the extremely slow growth rate in culture and almost complete lack of growth on agar suggests that this fungus has highly specific growth requirements and can be regarded as an obligate biotroph. The fungus barely grows in culture, attaining a colony diameter of only 1 mm after 1 month of incubation. Furthermore, this growth was a black, amorphous mass of sterile mycelium, which can hardly be regarded as a colony. This extremely slow growth rate is often reported in important leaf spotting fungi within *Capnodiales* (Crous *et al*., 2008), so *P. chamaeropicola* represents a new record of a phytopathogenic fungus. The report of a previously undescribed leaf spotting fungus in *Mycosphaerellales* represents a significant advance in the knowledge on TLD and MLD, since these fungi are important phytopathogens in various plant hosts, including *Eucalyptus* (Hunter *et al*., 2006; Crous *et al*., 2009a; Pérez *et al*., 2009; 2013; Taylor *et al*., 2012; Quaedvlieg *et al*., 2014). Furthermore, several species within *Mycosphaerellales* families, especially *Teratosphaeriaceae*, are of quarantine importance in many countries in Europe (Crous *et al*., 2009a; Quaedvlieg *et al*., 2012; Crous *et al*., 2019).

The present study has revealed a new disease of the ornamental, indigenous palm, *C. humilis*, caused by a fungal species in a previously unknown genus in the *Mycosphaerellales*. The geographical distribution of this fungus is, for now, confined to a single plant in the Lisbon district of Portugal. The survey conducted in this study can only be regarded as preliminary and it is likely that wider surveys will reveal more cases of this previously undescribed disease. Therefore, further sampling is essential to understand the geographical and ecological range of *P. chamaeropicola*. Future studies should also aim to elucidate the ecology and physiology of *Palmeiromyces* to assess its traits as a phytopathogen.

ACKNOWLEDGEMENTS

Work supported by UIDB/04046/2020 and UIDP/04046/2020 Centre grants from FCT, Portugal (to BioISI).

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