

Contribution to the phylogeny and a new species of *Coccodiella* (Phyllachorales)

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Abstract *Coccodiella* is a genus of plant-parasitic species in the family Phyllachoraceae (Phyllachorales, Ascomycota), i.e., tropical tar spot fungi. Members of the genus *Coccodiella* are tropical in distribution and are host-specific, growing on plant species belonging to nine host plant families. Most of the known species occur on various genera and species of the Melastomataceae in tropical America. In this study, we describe the new species *C. calatheae* from Panama, growing on *Calathea crotalifera* (Marantaceae). We obtained ITS, nrLSU, and nrSSU sequence data from this new species and from other freshly collected specimens of five species of *Coccodiella* on members of Melastomataceae from Ecuador and Panama. Phylogenetic analyses allowed us to confirm the placement of *Coccodiella* within Phyllachoraceae, as well as the monophyly of the genus. The phylogeny of representative species within the family Phyllachoraceae, including *Coccodiella* spp., gramminicolous species of *Phyllachora* and taxa with erumpent to superficial stroma from several host families, suggests that the genus *Phyllachora* might be polyphyletic. Furthermore, tar

spot fungi with superficial or erumpent perithecia seem to be restricted to the family Phyllachoraceae, independently of the host plant. We also discuss the biodiversity and host-plant patterns of species of *Coccodiella* worldwide.

Keywords *Coccodiella calatheae* · Phyllachoraceae · Plant parasites · Tar spot fungi · *Phyllachora* · Marantaceae · Zingiberales

Introduction

Hara (1911) introduced the genus *Coccodiella* for a plant-parasitic species characterized by a stroma originating in the mesophyll, which then proliferates through the lower epidermis, forming a sessile hypostroma attached to the host tissue. The sessile hypostroma then gives rise to an erumpent black epistroma, mainly superficial, flattened to pulvinate, uni- to multiloculate, and with a perithecia immersed in the epistroma. The asci are cylindrical to clavate while the ascospores are aseptate, globose to ellipsoid, and hyaline, becoming brown when mature with or without a gelatinous sheath. In the original description of *Coccodiella*, Hara (1911) erroneously characterizes the continuous ascospores as biseptate. Although later, Hara (1912) stated that the ascospores are not biseptate, the emendation was widely overlooked by other authors. Therefore, Theissen and Sydow (1914) erected the genus *Coccostroma* for the same fungi with non-septate ascospores. This genus has been partly revised by Arx and Müller (1954) based on a revision of the synonymous genus *Bagnisiopsis* Theiss. & Syd. (Theissen and Sydow 1915; Petrak 1928; Miller and Burton 1943). The genus *Coccodiella*, however, was treated as a genus with species, with septate ascospores as a completely different systematic context within the Dothideaceae, by Theissen and

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Sydow (1915) and Clements and Shear (1931). Petrak (1927, 1928) concluded that the genus is closely related to *Phyllachora*. Finally, Katumoto (1968) noticed the synonymy and transferred all the relevant known species to *Coccodiella*. Some *Coccodiella* species have been connected with an asexual form belonging to the genus *Hemidothis* Syd. & P. Syd. (Sydow and Sydow 1916; Petrak 1927, 1929; Seixas et al. 2007, Alves et al. 2014), which have been observed occurring in the stromata of *Coccodiella* spp.

Members of the genus *Coccodiella* are biotrophic parasites specific to their hosts, at least on the family level. This strong host specificity was demonstrated for the species *Coccodiella miconiae* (Duby) Hino and Katumoto and its host plant *Miconia calvescens* (Schr. & Mart.) DC (Alves et al. 2014). The 21 known *Coccodiella* spp. occur on plant species belonging to nine host plant families (Table 1). *Coccodiella arundinaria* Hara, found on bamboos in Japan, is the type species in this genus, but most species of *Coccodiella* occur on living leaves of various genera and species of Melastomataceae in tropical America (Miller and Burton 1943).

According to recent literature, the genus *Coccodiella* forms part of the order Phyllachorales and the family Phyllachoraceae, being closely related to *Camarotella* spp. with erumpent stroma and gramminicolous *Phyllachora* spp. with immersed stroma (Mardones et al. 2017). The main differences between *Coccodiella* and *Phyllachora* are the structure of the stroma and the host plant. *Phyllachora* spp. produce perithecia immersed beneath a pseudostromatic clypeus and always occur on members of the host family Poaceae (Mardones et al. 2017), while *Coccodiella* spp. produce erumpent to superficial epistromata that contain the perithecia and appear on a broad range of host families (Cannon 1991).

The main objective of the present study is to describe a new species of *Coccodiella* collected in Panama on *Calathea crotalifera* (Marantaceae). As sequences of several *Coccodiella* spp. on Melastomataceae are now available, we also used them to improve the genus concept of *Coccodiella*. Additionally, we discuss the biodiversity and host-plant patterns of species of *Coccodiella* known worldwide.

Material and methods

Collection and preservation of material Leaves of host plants infected with *Coccodiella* spp. were collected during several field trips in Ecuador and Panama between 2005 and 2016. Leaves bearing black stromata were dried in a plant press. Dried specimens collected in Ecuador were deposited at the Herbario de la Universidad Técnica Particular de Loja (HUTPL). Specimens from Panama were deposited at the Herbario de la Universidad de Panama (PMA) and the Herbario de la Universidad de Chiriqui (UCH). Isotypes of

all collected species were deposited in the Botanische Staatssammlung, München (M), Germany.

Morphology Microscopical examination was carried out using a Nikon Eclipse 80i microscope with bright field and phase contrast optics. Permanent preparations of stromatic tissues (stromata and perithecia) were made by sectioning tissue samples with a freezing microtome (Leica CM 1510-1), then mounting them in lactophenol with cotton blue. Semi-permanent preparations of asci, paraphyses, and ascospores were made using the following solutions: distilled water, 3% potassium hydroxide (KOH), and cotton blue (1 mg/mL). After pre-treatment of dried material with water, Melzer's reagent was used to test iodine color reactions of asci. Drawings were done using a drawing tube adapted to the microscope and edited with Photoshop CS5 (Adobe, San Jose, California). Microscopic photographs and measurements were made using a Nikon DS-Fi2 camera adapted to the microscope and operated by the Imaging Software NIS-Elements D 2.2. Thirty asci, ascospores, and other structures were measured in each collection, and a representative range was depicted. If less than 30 values were measured, the number is indicated in brackets. Measurements are given as ranges of values rounded to the nearest half micrometer. Extreme values are added in brackets.

DNA extraction, PCR amplification, and DNA sequencing

DNA extraction and PCR protocols followed the procedure described by Mardones et al. (2017). In short, universal primers ITS5/ITS4 were used for the ITS region amplification (White et al. 1990), primers NL1/NL4 (O'Donnell 1993) for the nrLSU DNA amplification, and primers NS1/NS4 (White et al. 1990) for the nrSSU DNA amplification.

Sequence alignment and model determination Sequences included in the phylogenetic analyses were either generated in this study, in recent studies on Phyllachorales (Mardones et al. 2017; Dos Santos et al. 2016) or retrieved from GenBank. The taxa of Phyllachorales used in the analyses and the newly generated sequences are deposited in GenBank under the accession numbers given in Table 2 together with their location and host plant.

We assembled a concatenated three-locus dataset for phylogenetic analyses, including the sequences of 33 specimens (Table 2). Since previous studies confirmed the placement of the genus *Coccodiella* in the family Phyllachoraceae (Mardones et al. 2017), the original matrix corresponding to this family was used as a template to include the newly sequenced species and the taxa retrieved from GenBank. Taxa belonging to the other two families in the Phyllachorales were removed, and two outgroup species were chosen to represent the family Telimenaceae (*Telimenia bicincta* and *T. picramniae*). Our sample series includes new sequences

Table 1 Known distribution and host plants of known species of *Coccodiella* (Phyllachorales, Ascomycota)

Host Family	Species of <i>Coccodiella</i>	Host Genera	Geographic Region	WWF Global Ecoregions ^a	Reference	
Asteraceae	<i>C. advena</i>	<i>Gynoxys</i>	Colombia	Neotropic	Chardon and Toro (1930)	
	<i>C. andicola</i>	<i>Verbesina</i>	Ecuador	Neotropic	Petrak (1950)	
Capparaceae	<i>C. capparidis</i>	<i>Capparidis</i>	India, Pakistan	Indo-malaya	Mundkur and Ahmad (1946)	
Dicksoniaceae	<i>C. nuda</i>	<i>Dicksonia</i> <i>Cibotium</i>	Hawaii	Oceania	Stevens (1925)	
	<i>C. polymorpha</i>	<i>Dicksonia</i> <i>Cibotium</i>	Hawaii	Oceania	Stevens (1925)	
Fabaceae	<i>C. machaerii</i>	<i>Machaerium</i>	Brazil	Neotropic	Arx and Müller (1954)	
Marantaceae	<i>C. calathea</i>	<i>Calathea</i>	Panama	Neotropic	This study	
Melastomataceae	<i>C. bullosa</i>	<i>Miconia</i>	Brazil	Neotropic	Petrak (1928)	
	<i>C. depressa</i>	<i>Miconia</i>	Brazil	Neotropic	Petrak (1928)	
	<i>C. leandrae</i>	<i>Leandra</i> <i>Miconia</i>	Brazil	Neotropic	Miller and Burton (1943)	
	<i>C. melastomatum</i>	<i>Clidemia</i> <i>Miconia</i> <i>Tibouchina</i>	South and Central America and Caribbean	Neotropic	Trampe (2010, and citations therein)	
	<i>C. miconiae</i>	<i>Clidemia</i> <i>Miconia</i>	South and Central America	Neotropic	Trampe (2010, and citations therein)	
	<i>C. miconiicola</i>	<i>Graffenrieda</i> <i>Miconia</i> <i>Ossaea</i>	South and Central America	Neotropic	Trampe (2010, and citations therein)	
	<i>C. minuta</i>	<i>Miconia</i>	Costa Rica	Neotropic	Miller and Burton (1943)	
	<i>C. neurophila</i>	<i>Clidemia</i> <i>Miconia</i> <i>Tibouchina</i>	South and Central America	Neotropic	Miller and Burton (1943)	
	<i>C. peribebuyensis</i>	<i>Miconia</i> <i>Monochaetum</i> <i>Tetrazygia</i>	South and Central America and Caribbean	Neotropic	Trampe (2010, and citations therein)	
	<i>C. toledoii</i>	<i>Clidemia</i> <i>Heterotrichium</i> <i>Miconia</i>	South America and Caribbean	Neotropic	Miller and Burton (1943)	
	<i>C. translucens</i>	<i>Miconia</i>	Venezuela	Neotropic	Miller and Burton (1943)	
	Myrtaceae	<i>C. myrtacearum</i>	<i>Campomanesia</i>	Brazil	Neotropic	Arx and Müller (1954)
	Poaceae	<i>C. arundinariae</i>	<i>Arundinaria</i>	China	Indo-malaya	Cannon (1996)
			<i>Dendrocalamus</i>	Japan		
			<i>Phyllostachys</i>			
<i>Pleioblastus</i>						
<i>Pseudosasa</i> <i>Sasa</i>						
Proteaceae	<i>C. banksiae</i>	<i>Banksia</i>	Australia	Australasia	Pearce et al. (2001)	
Rubiaceae	<i>C. puttemansii</i>	<i>Alibertia</i>	Brazil	Neotropic	Arx and Müller (1954)	

The species described in this paper is denoted in bold

^a According to Olson et al. (2001)

obtained from 11 specimens of *Coccodiella* corresponding to six species. In addition to *Coccodiella* spp. sequences, we also provide new sequence data for *Phyllachora* spp. on grasses and some other tar spot fungi with erumpent to superficial stroma which are closely related to *Coccodiella* spp. according to preliminary analyses (data not show). All the specimens that have been collected, identified and sequenced by us were compared with the corresponding type specimen to ensure that they exhibited the same morphological characteristics.

Sequence assembly and editing were performed using Geneious v7.1.9 (Kearse et al. 2012). Alignments for each gene and the concatenated dataset were performed by MAFFT v.7.164b (Kato and Standley 2013) using the L-INS-i algorithm. The program Gblocks 0.91b (Talavera and Castresana 2007) was used to remove poorly aligned positions and divergent regions of the DNA alignment using the parameters for a less stringent selection. The alignments were deposited in TreeBASE (<http://www.treebase.org/>) under accession number 21322.

Table 2 Specimen data of species of Phyllachorales included in the phylogenetic analyses. Accession numbers written in **bold** refer to sequences generated during the present study

Species	Voucher	Host	Host Family	Locality	GenBank Accession Numbers		
					nrLSU	nrSSU	ITS
<i>Camarotella costaricensis</i>	MM-149	<i>Acrocomia aculeata</i>	Arecaceae	Panama	KX430484	KX451863	KX451913
<i>Camarotella costaricensis</i>	MM-21	<i>Acrocomia aculeata</i>	Arecaceae	Panama	KX430490	KX451851	KX451900
<i>Camarotella</i> sp. 1	MM-142	<i>Licania arborea</i>	Chrysobalanaceae	Panama	KY907075	MF460375	KY907079
<i>Camarotella</i> sp. 2	MM-27	Unknown	Arecaceae	Panama	KX430492	KX451852	KX451901
<i>Cocodiella calathea</i>	MP5133	<i>Calathea crotalifera</i>	Marantaceae	Panama	MF460370	MF460376	MF460366
<i>Cocodiella</i> cf. <i>leandrae</i>	MM-152	<i>Miconia</i> sp.	Melastomataceae	Ecuador	MF460371	MF460377	MF460367
<i>Cocodiella melastomatum</i>	CMU78543	<i>Miconia</i> sp.	Melastomataceae	Venezuela	–	U78543	–
<i>Cocodiella miconiae</i>	ppMP1342	<i>Miconia</i> sp.	Melastomataceae	Panama	KX430506	KX451871	MF460365
<i>Cocodiella miconiicola</i>	TH571	<i>Ossaea micrantha</i>	Melastomataceae	Panama	KX430512	KX451880	–
<i>Cocodiella miconiicola</i>	TT077NL	<i>Ossaea micrantha</i>	Melastomataceae	Panama	KY907074	–	–
<i>Cocodiella miconiicola</i>	CBMAP-H290A	<i>Miconia</i> sp.	Melastomataceae	Panama	MF460373	MF460379	MF460368
<i>Cocodiella miconiicola</i>	CBMAP-H302B	<i>Miconia</i> sp.	Melastomataceae	Panama	MF460372	MF460378	–
<i>Cocodiella miconiicola</i>	SO-15	<i>Graffenrieda</i> sp.	Melastomataceae	Ecuador	MF460374	MF460380	MF460369
<i>Cocodiella toledo</i>	MM-165	<i>Miconia</i> sp.	Melastomataceae	Ecuador	KX430488	KX451865	KX451917
<i>Cocodiella toledo</i>	Unknown	<i>Miconia</i> sp.	Melastomataceae	Venezuela	–	U78544	–
<i>Phyllachora cerradensis</i>	UB15626	<i>Myrcia racemulosa</i>	Myrtaceae	Brazil	–	KC683454	–
<i>Phyllachora graminis</i>	RoKi3084	<i>Arrhenatherum elatius</i>	Poaceae	Germany	KX430507	KX451872	–
<i>Phyllachora graminis</i>	MM-166	<i>Hordelymus europaeus</i>	Poaceae	Germany	KX430503	KX451869	KX451920
<i>Phyllachora graminis</i>	DAOM240981	Unknown	Poaceae	Canada	–	HQ317550	–
<i>Phyllachora graminis</i>	Unknown	Unknown	Poaceae	Unknown	–	AF257111	–
<i>Phyllachora maydis</i>	BPI893231	<i>Zea mays</i>	Poaceae	USA	–	KU184459	–
<i>Phyllachora myrciae</i>	UB16930	<i>Myrcia variabilis</i>	Myrtaceae	Brazil	–	KC683456	–
<i>Phyllachora myrciariae</i>	UB21781	<i>Myrciaria delicatula</i>	Myrtaceae	Brazil	–	KC683469	–
<i>Phyllachora</i> sp. 1	MM-78	<i>Chusquea</i> sp.	Poaceae	Costa Rica	KX430498	KX451853	–
<i>Phyllachora</i> sp. 1	MM-98	<i>Chusquea longifolia</i>	Poaceae	Costa Rica	KX430502	KX451856	–
<i>Phyllachora</i> sp. 1	SO-07	<i>Chusquea</i> sp.	Poaceae	Ecuador	KX430517	KX451890	–
<i>Phyllachora</i> sp. 2	RMB1061	<i>Panicum maximum</i>	Poaceae	Benin	KX430504	KX451870	KX451921
<i>Phyllachora</i> sp. 3	MM-128	Bambusoideae	Poaceae	Thailand	KX430475	KX451859	KX451908
<i>Phyllachora</i> sp. 3	MM-129	Bambusoideae	Poaceae	Thailand	KX430476	KX451860	KX451909
<i>Phyllachora subcircinans</i>	UB09748	<i>Psidium australe</i>	Myrtaceae	Brazil	–	KC683441	–
<i>Phyllachora truncatispora</i>	UB14083	<i>Myrcia camapuensis</i>	Myrtaceae	Brazil	–	KC683448	–
<i>Telimena bicincta</i>	MM-133	<i>Picramnia antidesma</i>	Picramniaceae	Costa Rica	KX430478	KX451861	KX451910
<i>Telimena picramniae</i>	MM-05	<i>Picramnia</i> sp.	Picramniaceae	Panama	KX430470	KX451848	KX451896

The Congruence Among Distance Matrices test (CADM, Legendre and Lapointe 2004) was performed using patristic distance matrices to test the level of congruence among loci. Because no supported incongruence was detected, we conducted phylogenetic analysis based on the concatenated three-locus dataset.

The best-fit model of DNA evolution TrNef + I + G was applied for the ITS dataset, TrN + G model for the nrLSU dataset, and TrNef + G for the nrSSU dataset, following Akaike Information Criterion (AIC) as implemented in PartitionFinder 1.1.1 (Lanfear et al. 2012).

Phylogenetic analyses Molecular-phylogenetic analyses were performed using the Maximum likelihood (ML) and Bayesian inference (BI) approaches. The ML analyses were performed in RAxML (Stamatakis 2014) implemented in raxmlGUI v.0.9b2 (Silvestro and Michalak 2012) with 1000 non-parametric bootstrap iterations using the GTRGAMMA model and a discrete gamma distribution (Stamatakis et al. 2008). Bayesian analyses were carried out with the program MrBayes v. 3.2.6 (Ronquist et al. 2012) on XSEDE (Miller et al. 2010) on the CIPRES Science Gateway web portal (http://www.phylo.org/sub_sections/portal/). Two parallel

runs with eight chains of Metropolis-coupled Markov chain Monte Carlo iterations were performed. Analyses were run for 100 million generations, with trees sampled every 1000th generation. Burn-ins were determined by checking the likelihood trace plots in Tracer v1.6 (Rambaut et al. 2014) and subsequently discarded. To confirm the convergence of trees, the average standard deviation of split frequencies was monitored to ensure that it fell below 0.01 and log files from the Bayesian analyses were analyzed with Tracer and the online version of AWTY (Nylander et al. 2008). No indication of lack of convergence was detected. Bayesian posterior probabilities (BPP) ≥ 0.95 and Bootstrap values (BS) ≥ 70 were considered to be significant.

Results

Phylogenetic analyses

Individual alignment datasets for each marker consisted of 24 sequences/399 base pairs for ITS, 23/550 for nrLSU and, 24/944 for nrSSU. Individual dataset analyses (ITS, LSU, and SSU) yielded similar trees that differed only in interspecies relationships (data not shown). The combined dataset consisted of 33 fungal specimens representing 21 taxa within Phyllachoraceae, and had an aligned length of 1893 characters (857 characters were constant, and 1036 were variable). The ML and BI analyses of the concatenated dataset resulted in similar tree topologies, as well as support values for nodes. Figure 1 presents the ML phylogenetic tree.

The family Phyllachoraceae is strongly supported (100/1.0). Within this family, four major lineages can be distinguished. Lineage I (98/1.0) contains the type species *Phyllachora graminis* and *Ph. maydis*. The second lineage (99/1.0) includes some graminicolous species of *Phyllachora* s. l. with immersed perithecia on *Chusquea* spp. and tar spot fungi on host family Myrtaceae with subepidermal or intradermal perithecia. The only disagreement between BI and ML analyses is regarding this clade. In the ML analysis *Phyllachora* spp. on *Chusquea* spp. are in a subclade separate from *Phyllachora* spp. on Myrtaceae. In contrast, in the BI analysis all the species group in the same clade. A third lineage is represented in our tree by *Camarotella* sp. 1 growing on *Licania arborea* with low support. The fourth lineage includes a strongly supported subclade (85/0.99) formed by members of the genera *Camarotella* on Areaceae and *Coccodiella*. Closely related to this subclade, but without support, are two species of *Phyllachora* s.l. on bamboo. The relationships among these clades are mostly unresolved, with low to moderate posterior probability and bootstrap values.

Species of *Coccodiella* form a strongly supported monophyletic group (100/1.0). This clade received statistical support by all three individual datasets confirming its monophyly.

Coccodiella spp. are separated into three groups: a single branch representing *C. miconiae*, a group containing the new species *C. calatheae* on *Calathea crotalifera* (Marantaceae) and a specimen (MM-152) possibly representing *C. leandrae*; and a third group form by *C. melastomatum*, *C. miconiicola*, and *C. toledo*, infecting species of several genera of Melastomataceae.

Taxonomy

Coccodiella calatheae Mardones & M. Piepenbr., **sp. nov.**

Mycobank no.: MB821977; Figs. 2 and 3.

Etymology: *calatheae* refers to the host genus of this species, *Calathea*.

Type: Panama, Chiriquí province, Reserva Forestal Fortuna, Bijao, on *Calathea crotalifera* S. Watson (Marantaceae), leg. M. Piepenbring, D. Cáceres, A. Krohn and J. Villegas 5133, 02.08.2012. Holotype (PMA), Isotype (M-0141297).

Stroma conspicuous, hypophyllous, gregarious, discrete, scattered all over the leaf blade, erumpent, subglobose to pulvinate, 0.7–1.2 mm diam., black, surrounded by pale brown discoloration zones which become darker when mature, also with less apparent yellowish spots on the side of the leaf opposite to the stromatic development. *Epistroma* superficial, multiloculate, mainly consisting of *textura angularis*, with two defined layers, an exterior layer formed by cells in about 2 or 3 rows and a very melanized inner layer, about 350–400 μm wide, formed by pale brown to hyaline cells. *Hypostroma textura angularis*, pale brown to hyaline, 50–75 μm wide, developing a stromatic foot within the host tissue, which expands through the leaf, partly reaching the abaxial side of the leaf. *Perithecia* immersed in the epistroma, pyriform, 220–340 \times 125–250 μm ($n = 13$), necks 25–60 μm long ($n = 7$), wall 40–90 μm thick, melanized, well differentiated from stromatal tissue, with cells usually more pigmented in the outer layers. *Periphyses* present. *Paraphyses* filiform, hyaline, longer than asci, about 2 μm wide. *Asci* subglobose to saccate, sometimes cylindrical, 70–85 \times 12–16 μm ($n = 30$), with rounded apex, with inconspicuous ascus crown, I-, short-stalked, 8-spored. *Ascospores* usually biseriate, rarely uniseriate, ellipsoid to oblong, 9–12 \times 6–8 μm ($n = 30$), aseptate, hyaline, thin-walled, without a gelatinous sheath. *Asexual morph* not observed.

Notes: Species in *Coccodiella* are traditionally delimited based on host plant relationships and morphological characteristics of the epistroma and ascospores (shape and size). For the purpose of this study, *Coccodiella* spp. are considered at least specific on the level of host plant family.

For species of the order Zingiberales, eight species of tropical tar spot fungi are known, three for Marantaceae (*Phyllachora atrofingurans*, *Ph. goeppertiae* and *Ph. pulchra*), two for Strelitziaceae (*Ph. ravenalae* and *Ph. strelitziae*) and three for Zingiberaceae (*Ph. alpiniae*, *Ph. elettariae* and *Ph.*

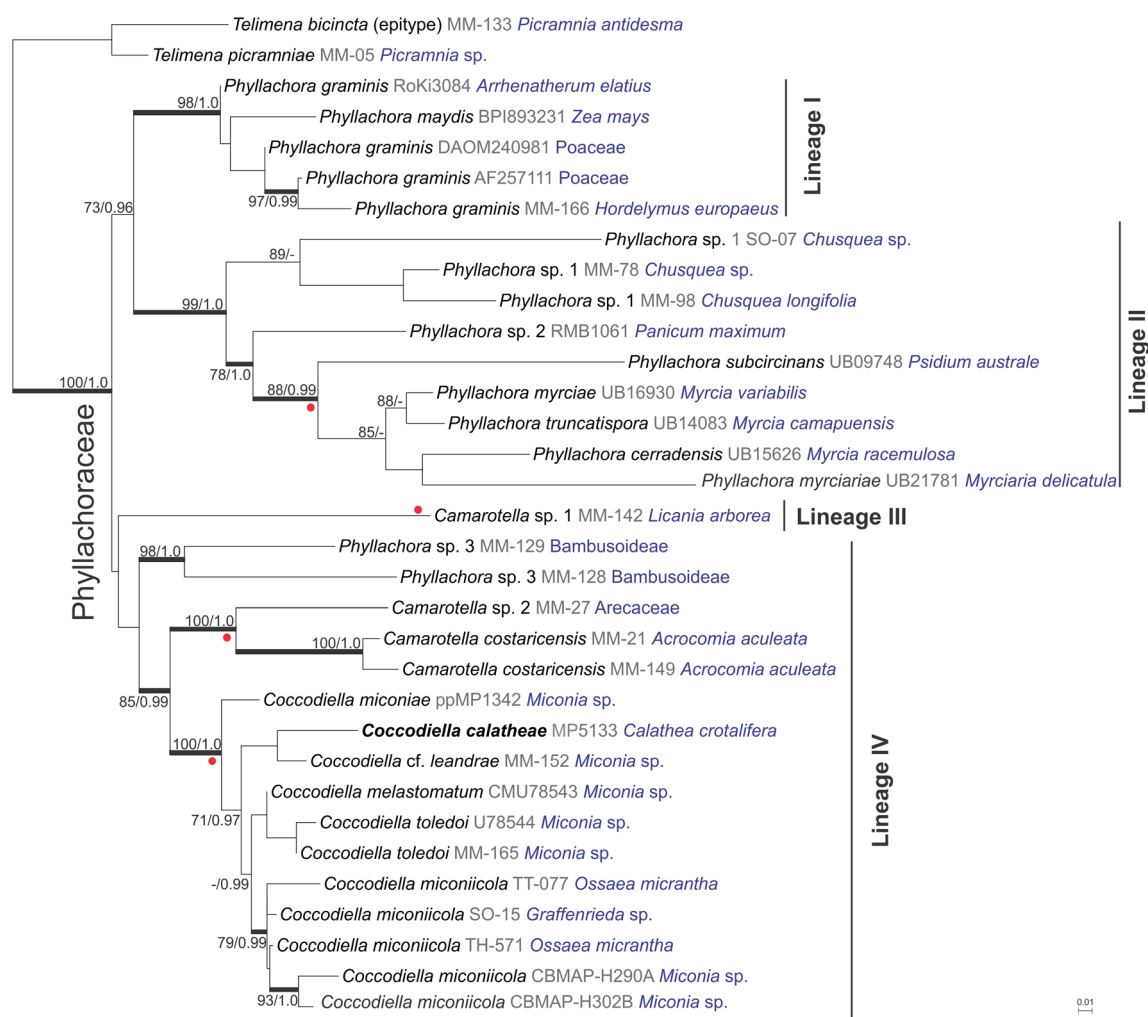


Fig. 1 Phylogenetic relationships within the family Phyllachoraceae. This is a maximum likelihood (ML) phylogeny based on three nuclear markers (nrLSU, nrSSU, ITS). Support values are ML bootstrap values based on 1000 replicates and posterior probabilities from a Bayesian analysis. ML bp > 70% and Bayesian PP > 0.95 are shown in the

internal branches. Internal branches considered strongly supported by both analyses are indicated by thickened branches. The name of the newly described species is written in bold. Information on host plants is written in blue text. Red dots indicate that the corresponding clade includes species with superficial or erumpent stromata

renealmiae). No species of *Coccodiella* is hitherto known from the host family Marantaceae and the order Zingiberales (Table 1).

Regarding morphological characteristics, the species *C. calatheae* is characterized by superficial and pulvinate epistromata with ellipsoid aseptate ascospores that are shorter than 12 μm . Several species of *Coccodiella* can be easily distinguished from *C. calatheae* by characteristics of the epistroma, i.e. by the presence of setae in *C. nuda* and *C. polymorpha* (Stevens 1925), appendages in *C. neurophila* and *C. toledo* (Miller and Burton 1943), conical projections representing spermogonia in *C. miconiae* (Seixas et al. 2007) or translucent epistroma with reduced melanin pigmentation in *C. minuta* and *C. translucens* (Miller and Burton 1943). When remaining *Coccodiella* spp. with ascospores shorter than 15 μm are compared, *C. calatheae* can be distinguished from *C. miconiae* and *C. minuta* because of their spherical

ascospores (Miller and Burton 1943; Arx and Müller 1954). *C. melastomatum* differs from *C. calatheae* by the cup-shaped epistroma with a plane surface and the almost spherical ascospores and *C. toledo* can be recognized by the surface projections in the epistroma. The species *C. leandrae* differs from the new species by irregular-applanate stromata and ovate ascospores.

Therefore, considering its host association and morphological characteristics, we propose the fungus from Panama as a new species.

Discussion

A list of the accepted species of *Coccodiella* with their corresponding host plants and known distribution worldwide is presented in Table 1. Species of *Coccodiella* are strictly

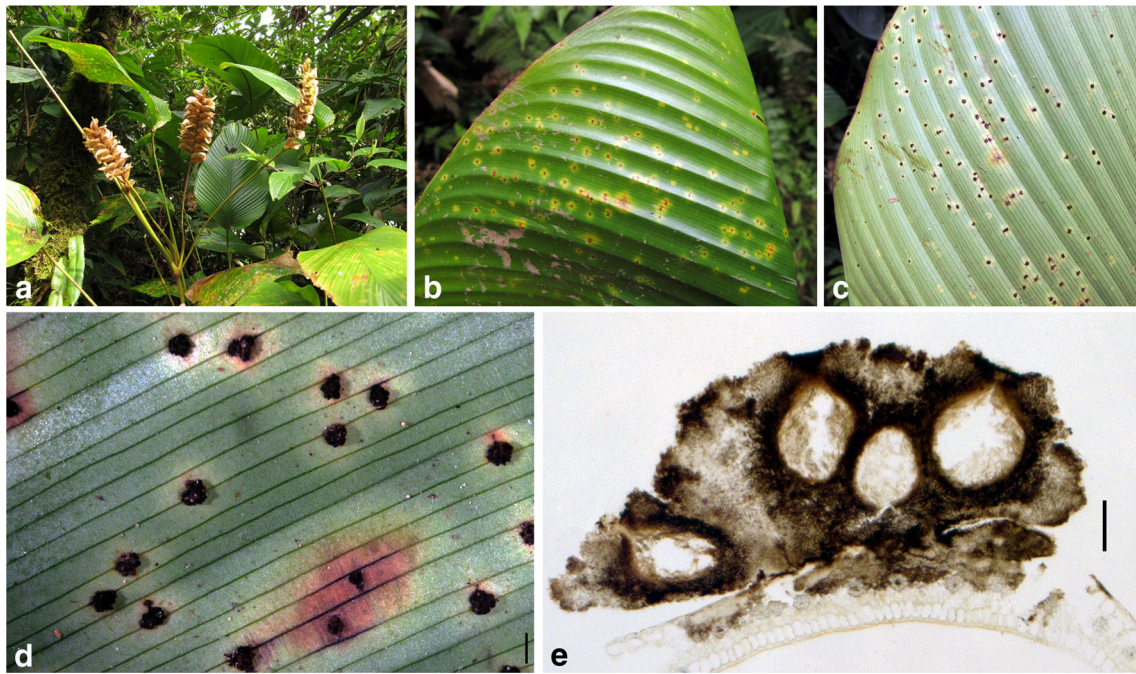


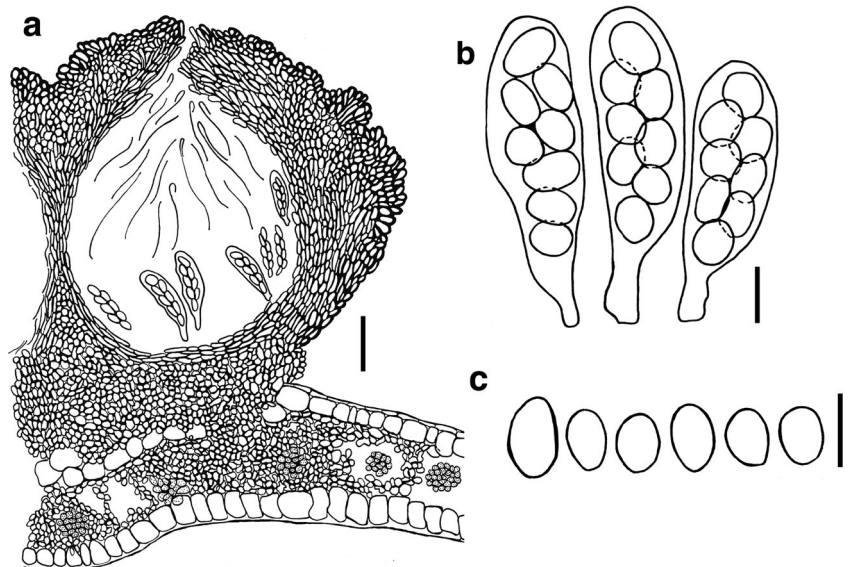
Fig. 2 *Coccidiella calatheae* on *Calathea crotalifera* (Marantaceae, MP5133). **a.** Infected plant from which the holotype originated; **b.** Upper surface of an infected leaf with discoloration zones; **c.** Underside of an

infected leaf with fruiting bodies; **d.** Numerous stomata growing on the underside of the leaf; **e.** Longitudinal section through the superficial stroma. Scale bars: d = 1 mm; e = 100 μ m

tropical in distribution. They are diversified on host plants that include ferns, monocotyledonous and dicotyledonous plants. The known species richness is higher in the Neotropics than for other parts of the world. However, the absence of records from Africa might reflect collection biases towards certain geographical regions. Some *Coccidiella* spp. are known to parasitize only one single host species, such as *C. banksiae* (Hansf.) Swart. on *Banksia marginata* Cav. (Proteaceae, Pearce et al. 2001), or are known only from the host of the type locality, such as the newly described species *C. calatheae* on *Calathea crotalifera*. Among the 22 known species of

Coccidiella, 11 have been reported on 30 species of the host tribe Miconiae in the family Melastomataceae, e.g., all the species have been recorded on species of *Miconia*, but most of them occur on a range of host species from at least three closely related genera (Petraik 1928; Miller and Burton 1943). The family Melastomataceae is among the most abundant and diversified groups of plants and is restricted to the New World (Goldenberg et al. 2008). These findings suggest an intimate relationship between neotropical Melastomataceae and the species diversification in the genus *Coccidiella*. Considering the strictly tropical distribution of *Coccidiella* spp. and the

Fig. 3 *Coccidiella calatheae* on *Calathea crotalifera* (Marantaceae, MP5133), as seen by light microscopy. **a.** Part of a longitudinal section of a superficial stroma, with one perithecium shown in detail. **b.** Asci with ascospores; **c.** Ascospores. Scale bars: a = 50 μ m; b = 15 μ m; c = 10 μ m



wide range of host plants, we expect both broader host ranges and a much greater number of species to exist.

Phylogenetic relationships and host relationships in the family Phyllachoraceae and the genus *Coccodiella*

Currently, three families are recognized in the order Phyllachorales: Phaeochoraceae, Phyllachoraceae and Telimenaceae (Mardones et al. 2017). Among members of Phyllachorales, the position of the perithecia varies from completely immersed in the mesophyll of the leaf to completely superficial. The presence of completely immersed perithecia has been suggested to be the ancestral state of Phyllachorales (Mardones et al. 2017). Tar spot fungi with immersed perithecia are present in the families Phyllachoraceae and Telimenaceae, and can be distinguished mainly by their host plant relationships. Species occurring on Poaceae belong only to Phyllachoraceae and species growing on the remaining monocotyledonous and eudicotyledonous host families belong to Telimenaceae (Mardones et al. 2017). This study, as well as a previous study (Mardones et al. 2017), has shown that independently of the host plant, tar spot fungi with superficial or erumpent perithecia seem to be restricted to the family Phyllachoraceae. Our results suggest that transitions between immersed to erumpent or superficial perithecia have taken place multiple times in Phyllachoraceae, such as in *Coccodiella* spp., *Camarotella* spp. on palms, and *Camarotella* sp. on *Licania arborea*. Recently sequenced taxa with subepidermal or epidermal perithecia growing on Myrtaceae (Dos Santos et al. 2016), are surprisingly placed in the Phyllachoraceae. Other genera with erumpent stromata, such as *Oxodeora* and *Coccostromopsis*, have been suggested to be closely related to *Coccodiella* and *Camarotella* (Hyde and Cannon 1999). However, no molecular data are available to corroborate this hypothesis.

Several authors noticed the polymorphic nature of *Coccodiella* spp., and suggested that several of the genera previously placed in synonymy may be re-established (Cannon 1991, Pearce et al. 2001). However, although not all known species of *Coccodiella* are represented in our molecular dataset, the fact that the six included taxa from a monophyletic group within Phyllachoraceae, allow us to suggest the monophyly of *Coccodiella*, including species with erumpent and superficial stromata and hyaline aseptate ascospores.

The present study provides insights into the evolution of a genus of biotrophic tar spot fungi. However, the genus *Coccodiella* remains in urgent need of revision. Currently available molecular data are not sufficient to elucidate relationships below genus level. Fresh material and more sequences of the remaining species of *Coccodiella*, including the type species, are necessary, as well as morphological examination of a higher number of specimens worldwide.

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