



# Phylogeny of the order *Phyllachorales* (*Ascomycota*, *Sordariomycetes*): among and within order relationships based on five molecular loci

M. Mardones<sup>1,2</sup>, T. Trampe-Jaschik<sup>1</sup>, S. Oster<sup>1</sup>, M. Elliott<sup>3</sup>, H. Urbina<sup>4</sup>,  
I. Schmitt<sup>1,5</sup>, M. Piepenbring<sup>1</sup>

## Key words

ancestral state reconstruction  
plant parasitic  
tar spot fungi  
*Telimenaceae*

**Abstract** The order *Phyllachorales* (*Pezizomycotina*, *Ascomycota*) is a group of biotrophic, obligate plant parasitic fungi with a tropical distribution and high host specificity. Traditionally two families are recognised within this order: *Phyllachoraceae* and *Phaeochoaraceae*, based mostly on morphological and host characteristics. Currently, the position of the order within the class *Sordariomycetes* is inconclusive, as well as the monophyly of the order, and its internal phylogenetic structure. Here we present a phylogeny of the order *Phyllachorales* based on sequence data of 29 species with a broad host range resulting from a wide geographical sampling. We inferred Maximum Likelihood and Bayesian phylogenies from data of five DNA regions: nrLSU rDNA, nrSSU rDNA, ITS rDNA, and the protein coding genes *RPB2*, and *TEF1*. We found that the order *Phyllachorales* is monophyletic and related to members of the subclass *Sordariomycetidae* within *Sordariomycetes*. Within the order, members of the family *Phaeochoaraceae* form a monophyletic group, and the family *Phyllachoraceae* is split into two lineages. Maximum Likelihood ancestral state reconstructions indicate that the ancestor of *Phyllachorales* had a monocotyledonous host plant, immersed perithecia, and a black stroma. Alternative states of these characters evolved multiple times independently within the order. Based on our results we redefine the family *Phyllachoraceae* and propose the new family *Telimenaceae* with *Telimenia erythrinae* as type species, resulting in three families in the order. Species of *Telimenia* spp. occur in several monocotyledonous and eudicotyledonous host plants except *Poaceae*, and generally have enlarged black pseudostroma around the perithecia, a character not present in species of *Phyllachoraceae*.

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## INTRODUCTION

*Phyllachorales* is an order of biotrophic, obligate plant parasitic fungi in the class *Sordariomycetes*, i.e., inoperculate pyrenomycetes. About 1 226 species are currently accepted in the order (Kirk et al. 2008), although 160 000 species have been estimated to occur worldwide (Cannon 1997). *Phyllachorales* are highly diverse in the tropics, relatively common in disturbed and natural vegetation, and likewise found in open and forested areas (Piepenbring et al. 2011).

Species of *Phyllachorales* are leaf- or stem-inhabiting micro-fungi with shiny black stromata, which gave them the common name ‘tropical tar spot fungi’. They are morphologically characterised by: deep black stromata of various shapes (except in species of *Polystigma* which have brightly coloured stromata); pseudostroma inside the host tissue and usually beneath an epidermal clypeus; perithecia usually strongly melanised that may be superficial, erumpent or immersed in the host tissue (Fig. 1a–f); thin-walled paraphyses which frequently deliquesce;

unitunicate asci of cylindrical to clavate shape, with an ascus crown and an inconspicuous apical ring not staining blue in iodine; and globose to filiform ascospores, which in most species are hyaline and 1-celled, with only a few genera including species with brown or septate ascospores (Parbery 1967, Cannon 1991, 1997). Although it has been difficult to connect asexual and sexual morphs in the order due to their obligately parasitic condition, some species of *Phyllachorales* have been linked to the asexual genus *Linochora* (Von Höhnel 1910), which may be inconspicuous and spermatial in function (Parbery & Langdon 1963, Parbery 1996).

Due to their biotrophic nutrition mode, high host specificity is assumed and species concepts are based partly on the identity and systematic position of the corresponding host plants. Hence to identify species of *Phyllachorales*, it is necessary to identify the host plant. Traditionally, new species have been described on the basis of new host records at generic level. However, examination of species of *Phyllachorales* on the host families *Poaceae* and *Fabaceae* demonstrated that tropical tar spot species are not restricted to a single host genus, but may occur on species belonging to a group of closely related genera (Parbery 1978, Cannon 1991, 1997). Species delimitation based mainly on host identity may therefore lead to over-splitting of species (Cannon 1997).

*Phyllachorales* are associated with diverse host plants, and most of the species are linked to angiosperms, with a few exceptions including the lichenicolous *Lichenochora* species, the marine algalicolous genus *Phycomelaina*, and some species on ferns and gymnosperms. Within the angiosperms, the following families are preferentially parasitized: *Arecaceae*, *Fabaceae*,

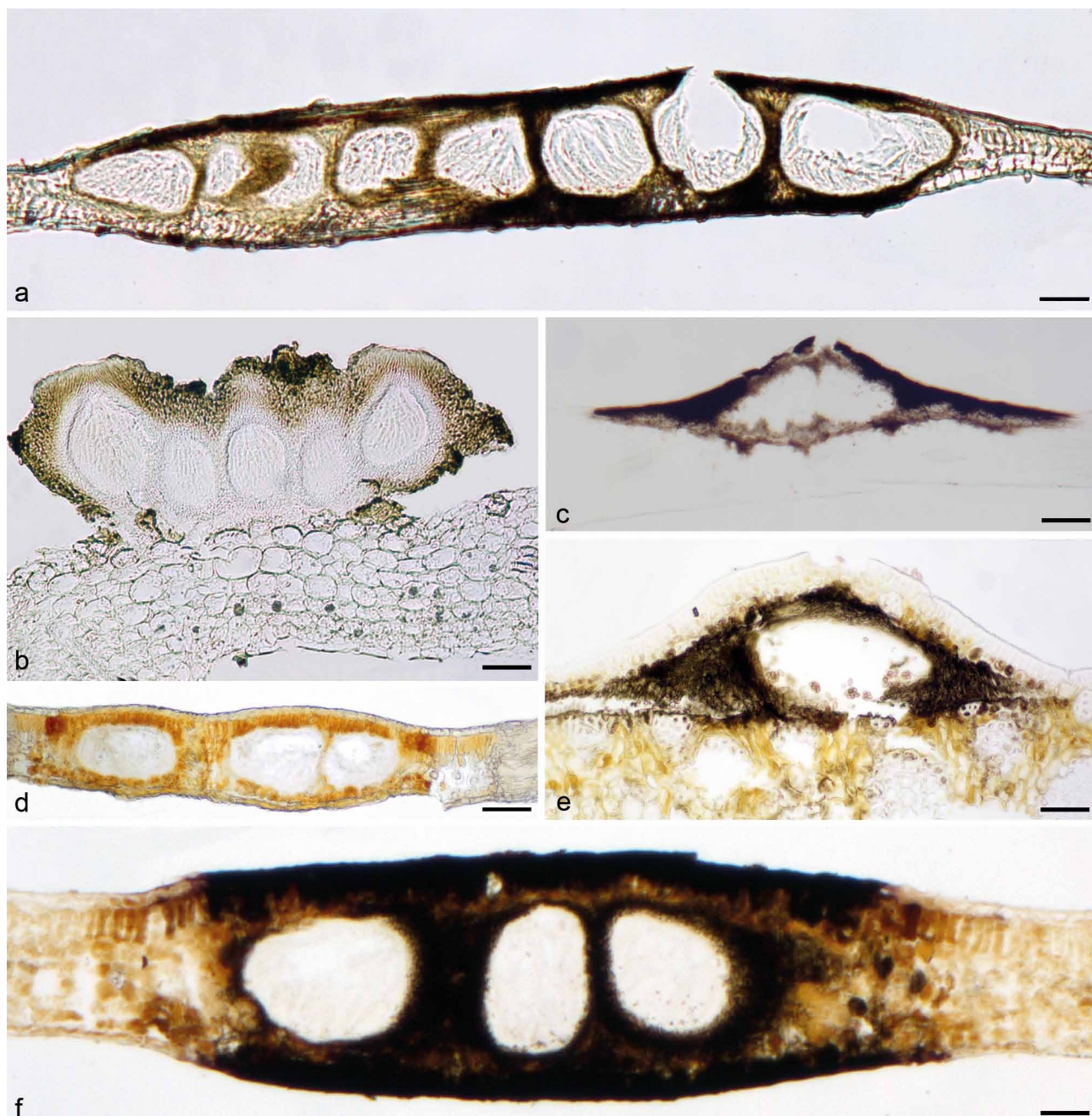
<sup>1</sup> Institute of Ecology, Evolution and Diversity, Faculty of Biosciences, Goethe University Frankfurt am Main, Biologicum, Max-von-Laue-Str. 13, 60439 Frankfurt am Main, Germany; corresponding author e-mail: melissamardones@gmail.com.  
<sup>2</sup> Escuela de Biología, Universidad de Costa Rica, San Pedro, 11501 San José, Costa Rica.  
<sup>3</sup> Department of Plant Pathology, University of Florida – IFAS, Fort Lauderdale Research and Education Center, Davie, FL-33314, USA.  
<sup>4</sup> Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN-47907, USA.  
<sup>5</sup> Senckenberg Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, 60325 Frankfurt am Main, Germany.

*Lauraceae*, *Melastomataceae*, *Moraceae*, *Myrtaceae*, and *Poaceae* (Cannon 1997). Monographs for phyllachoraceous species are available for *Arecaceae* (Hyde & Cannon 1999), *Fabaceae* (Cannon 1991), and *Poaceae* (Orton 1944, Parbery 1967). Additional host families studied include *Asclepiadaceae* (Pearce et al. 1999), *Erythroxylaceae* (Cannon & Evans 1999), *Proteaceae* (Pearce et al. 2001), and *Rosaceae* (Cannon 1996).

The genus *Phyllachora* was introduced on a herbarium label in Fuckels exsiccate series 'Fungi Rhenani' with a single species, *P. agrostis* (Fuckel 1867 in Cannon 1991), currently accepted as *Scirrhia agrostis* in *Dothideales* (Eriksson 1967). Later the genus *Phyllachora* was lectotypified with *Phyllachora graminis* as generic type (Clements & Shear 1931), and the genus name in the sense of Fuckel (1870) was conserved to allow continued use in its currently accepted circumscription. The order *Phyllachorales* was formally described by Barr (1983). However,

throughout the history of the group, several authors have placed phyllachoraceous fungi into various families and orders, stressing different morphological and ecological characteristics: *Diaporthales* (Cannon 1988), *Dothideales* (Saccardo 1876, Theissen & Sydow 1915), *Polystigmatales* or *Polystigmataceae* (Von Arx & Müller 1954, Eriksson 1982, Hawksworth et al. 1983), *Sphaeriales* (Nannfeldt 1932, Luttrell 1951, Müller & Von Arx 1962, 1973), and *Xylariales* (Barr 1983). For a detailed description of the taxonomical history of the order see Cannon (1991) and Pearce & Hyde (2006).

The order *Phyllachorales* comprises the families *Phyllachoraceae* and *Phaeochoraceae*. The family *Phyllachoraceae* was erected by Theissen & Sydow (1915) and is by far the largest family within the order with almost 1 200 described species (Kirk et al. 2008). The number of genera varies between 51 (Kirk et al. 2008) and 73 ([www.indexfungorum.org](http://www.indexfungorum.org)). Many of



**Fig. 1** Perithecia of species of *Phyllachorales* in different positions in the mesophyll of the leaves. a. *Phyllachora graminis* (isotype CUP3536) with immersed perithecia and few pseudostroma; b. *Coccodiella miconiae* (ppMP1342) with superficial perithecia; c. *Camarotella costaricensis* (MM-21) with erumpent perithecia; d. *Polystigma pusillum* (MM-113) with brightly coloured stroma; e. *Serenomyces phoenicis* (F59049) with subcuticular perithecia and without a clypeus; f. *Telimena bicincta* (epitype MM-133) with immersed perithecia and strongly developed pseudostroma. — Scale bars = 100 µm.



these genera, however, have less than ten species and 27 are monotypic. *Phyllachora* is the largest genus with 994 species; and *Coccodiella*, *Lichenochora*, *Ophiodothella*, *Polystigma*, and *Trabutia* are also large genera with 22, 40, 36, 24, and 35 species, respectively ([www.indexfungorum.org](http://www.indexfungorum.org)). The family *Phaeocharaceae* is a small group with 19 accepted species in the genera *Cocoicola*, *Phaeochora*, *Phaeochoropsis*, and *Serenomyces* and is known to occur only associated with species of *Arecaceae* (Hyde et al. 1997). *Phaeocharaceae* were provisionally assigned to *Phyllachorales* since the family was erected (Hyde et al. 1997), mainly due to their unusual stromatic characteristics. However, no molecular studies are available to clarify the family's phylogenetic placement.

Molecular phylogenetic studies including members of the *Phyllachorales* are infrequent, mainly because it is difficult to obtain cultures of these biotrophic fungi. The existing molecular studies indicate, without support and with a limited sampling, that the order might be related to *Sordariales* or *Boliniales* (Winka & Eriksson 2000, Wanderlei-Silva et al. 2003, Inderbitzin et al. 2004, Trampe 2010). A recent large-scale phylogenetic study confirms the position of *Phyllachorales* in the subclass *Sordariomycetidae* with high support (Maharachchikumbura et al. 2015). However, in this phylogeny based on four loci, *Phyllachorales* are represented only by three taxa and the single locus nrSSU.

The phylogeny within the order *Phyllachorales* is still almost unresolved and its monophyly has not yet been resolved. It is known that the *Glomerella/Colletotrichum* complex that was placed within *Phyllachorales* in the past, belongs to the *Glomerellales* (Wanderlei-Silva et al. 2003, Réblová et al. 2011) fungi. Some studies suggested that *Phyllachorales* are a polyphyletic assemblage, since several taxa had to be excluded from this order: *Ophiodothella* and *Sphaerodothis* were transferred to *Xylariales* and *Hypocreales*, respectively (Wanderlei-Silva et al. 2003); *Plectosphaera eucalypti* to *Xylariales* (Summerell et al. 2006), and *Polystigma amygdalinum* to subclass *Xylariomycetidae* (Habibi et al. 2015). These studies also suggested that among the studied taxa, *Phyllachora* and *Coccodiella* are the only genera forming a monophyletic clade, closely related to *Sordariales*, and considered as true *Phyllachorales*. Due to the limited availability of molecular phylogenetic data, information is lacking concerning the evolution of morphological traits and the co-evolution trails with the hosts.

The aims of this study are:

- 1 to confirm the phylogenetic position of *Phyllachorales* within *Sordariomycetidae*;
- 2 to determine the monophyly of *Phyllachorales*;
- 3 to define monophyletic clades within the order for the delimitation of families; and
- 4 to reconstruct the evolution of morphological and ecological characteristics to assess their value as systematic criteria.

To achieve these objectives, we inferred the first comprehensive multilocus phylogeny for the order *Phyllachorales*.

## MATERIALS AND METHODS

### Taxon sampling

Fresh specimens representing the two recognised families of *Phyllachorales* were collected mainly in Costa Rica during 2012–2015 and Western Panama during 2007–2015 (Trampe 2010). Additional specimens were collected from Benin, Ecuador, Germany, Thailand, and the USA. A total of 48 collections of tropical tar spot fungi, representing 29 species and six genera were sequenced. Specimens collected in the context of the present study were deposited in the following herbaria: FR, M, UCHI, USJ, and HUTPL.

### Extraction, amplification, and sequencing of DNA

DNA was isolated directly from hymenia of fresh, recently collected material or from dry specimens except for the species belonging to family *Phaeocharaceae*, which were available as cultures previously isolated by Elliott & Des Jardin (2014). To extract non-melanised cells with high quality DNA, for each stroma, the clypeus was cut off in half to exposed the hymenia of 1–10 perithecia (diam c. 0.2–0.5 mm) that were removed and placed into 1.5 mL sterilised microtubes containing Cetyltrimethyl ammonium bromide (2 % CTAB). The isolation of genomic DNA from fresh material was performed with 600 µL of extraction buffer (2 % CTAB; 100 mM Tris-HCl, pH 8; 1.4 M NaCl, and 20 mM EDTA) and the DNA was extracted using phenol-chloroform : isoamyl alcohol (24 : 1). For dry material the E.Z.N.A.® Forensic DNA Extraction Kit (VWR-Omega, USA) was used following the manufacturer's instructions with a few modifications. The material was homogenized for 5–10 min using a Retsch Mixer Mill MM301 with STL buffer and 2.5 mm Zirconia beads. Isolated DNA was resuspended in sterile water and stored at -20 °C. DNA concentration was checked by electrophoresis in 0.8 % agarose and by the spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific, USA). Several attempts were made to extract DNA from older herbarium specimens (type specimens) but they were unsuccessful. For that reason, the macro- and micro-morphology of extracted phyllachoraceous specimens were rigorously compared with the respective type specimen when it was possible.

Five partial nuclear gene regions (three ribosomal loci and two protein-coding genes) were amplified and sequenced: one fragment of the large subunit nuclear ribosomal DNA (nrLSU) with primers NL1 and NL2 (O'Donnell 1993), one fragment of the small subunit nuclear ribosomal DNA (nrSSU) with primers NS1 and NS4 (White et al. 1990), the complete internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2) with primers ITS5 and ITS4 (White et al. 1990), one fragment of the second largest subunit of RNA polymerase II (*RPB2*) with primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999), and one fragment of the translation elongation factor 1 (*TEF1*) with primers EF1-983f (Carbone & Kohn 1999) and EF1-2218r (Rehner & Buckley 2005). PCR reactions were performed on a PEQSTAR 2X GRADIENT Thermal Cycler (PEQLAB, Erlangen, Germany) using VWR Taq DNA polymerase (VWR-Omega, USA).

The reactions followed this protocol: Each 50 µL PCR mixture included 10 µL of 5× buffer, 3 µL (25 mM) of magnesium chloride (MgCl<sub>2</sub>), 0.8 µL (20 mM) of dNTP mix, 1 µL (10 mM) of each primer, 0.4 µL (5U/µL) of Taq Polymerase, 1 µL of template DNA, and 37.8 µL of sterile distilled water. For *RPB2* and *TEF1* 4 µL of each primer and 4 µL of template DNA were used, and the amount of water decreased accordingly. Conditions of the PCR for nrLSU, nrSSU, and ITS were as follows: DNA denaturation 94 °C for 4 min; 35 cycles of DNA denaturation 94 °C for 30 s, primer annealing 55 °C for 30 s and TAQ extension 72 °C for 45 s, and a final TAQ extension 72 °C for 5 min, followed by storage at 8 °C. Thermal cycling parameters of the *RPB2* and *TEF1* genes were performed as described by Liu et al. (1999) and Rehner & Buckley (2005), respectively.

PCR-products were checked on 1.5 % agarose electrophoresis gels stained with ethidium bromide. Amplified PCR products were purified with the Cycle Pure Kit (VWR-Omega, USA). The sequencing in both directions was performed with the same PCR primers with an Applied Biosystems 3730 DNA Analyzer in the BiK-F Laboratory Centre, Frankfurt am Main, Germany.

### Sequence alignment and model determination

Alignments for each gene were made by MAFFT v. 7.164b (Katoh & Standley 2013) using the L-INS-i algorithm, and adjusted manually using Mega v. 6.06 (Tamura et al. 2013). The

program Gblocks v. 0.91b (Talavera & Castresana 2007) was used to remove poorly aligned positions and divergent regions from the DNA alignment using the parameters for a less stringent selection.

Among the acquired sequences, often multiple sequences were recovered from a single stroma for a given genetic locus. To distinguish DNA of contaminants from phyllachoraceous DNA, all sequences were subjected to BLAST searches to verify the identity, and preliminary phylogenetic analyses were also performed including common plant parasitic fungi in *Pezizomycotina*.

To test the level of congruence among loci, the Congruence Among Distance Matrices test (CADM, Legendre & Lapointe 2004) was performed using patristic distance matrices to test the null hypothesis of complete incongruence among loci. This analysis has been shown to have an accurate type-I error rate (Campbell et al. 2011).

We assembled two datasets for phylogenetic analyses: a three-locus concatenated alignment (nrSSU, nrLSU, *RPB2*) including 88 specimens representing the three recognized subclasses of the *Sordariomycetes* following the classification of Zhang et al. (2006). The purpose of this analysis was to infer the position of *Phyllachorales* within *Sordariomycetes*, and to test the monophyly of the order. Sequences of further representative species of orders in *Sordariomycetes* were downloaded from GenBank (Table 1) mostly from studies published by Spatafora et al. (2006) and Zhang et al. (2006). *Leotia lubrica* was selected as outgroup taxon based on Zhang et al. (2006), and missing data were shown as gaps.

The second dataset is a four-locus concatenated alignment (nrSSU, ITS, *RPB2*, *TEF1*) including 51 specimens representing members of the order *Phyllochorales*. The alignment contained mostly sequences generated in this study plus all sequences of the *Phyllachorales* available from GenBank. The purpose of

**Table 1** Sequences downloaded from GenBank (in alphabetical order) used in this study.

Species	Order	Source	GenBank Accession Numbers			Reference
			nrLSU	nrSSU	<i>RPB2</i>	
<i>Aniptodera chesapeakeensis</i>	<i>Microascales</i>	ATCC 32818	U46882	U46870	DQ470896	Spatafora et al. (2006)
<i>Balansia henningsiana</i>	<i>Hypocreales</i>	AEQ96-27a	AY489715	AY489683	DQ522413	Spatafora et al. (2006)
<i>Bionectria ochroleuca</i>	<i>Hypocreales</i>	AFTOL-ID 187	DQ862027	DQ862044	DQ862013	Zhang et al. (2006)
<i>Bombardia bombardae</i>	<i>Sordariales</i>	SMH 3391	DQ470970	DQ471021	DQ470923	Spatafora et al. (2006)
<i>Buergeriella spartinae</i>	<i>Magnaporthales</i>	ATCC 22848	DQ341492	DQ341471	–	Thongkantha et al. (2009)
<i>Calosphaeria pulchella</i>	<i>Calosphaeriales</i>	CBS 115999	AY761075	AY761071	GU180661	Réblová & Seifert (2004)
<i>Camarops amorphae</i>	<i>Boliales</i>	SMH1450	AY780054	–	AY780156	Miller & Huhndorf (2005)
<i>Camarops microspora</i>	<i>Boliales</i>	CBS 649.92	AY083821	DQ471036	DQ470937	Spatafora et al. (2006)
<i>Camarops tubulina</i>	<i>Boliales</i>	SMH4614	AY346266	–	AY780157	Miller & Huhndorf (2005)
<i>Camarops ustulinoides</i>	<i>Boliales</i>	DEH 2164	DQ470941	DQ470989	DQ470882	Spatafora et al. (2006)
<i>Cercophora coprophila</i>	<i>Sordariales</i>	SMH3794	AY780058	–	AY780162	Miller & Huhndorf (2005)
<i>Chaetosphaerella phaeostroma</i>	<i>Coronophorales</i>	SMH4257	AY695264	–	FJ968940	Huhndorf et al. (2004)
<i>Chrysosporthe cubensis</i>	<i>Diaporthales</i>	CBS 101281	AF408338	DQ862047	DQ862016	Zhang et al. (2006)
<i>Cordyceps cardinalis</i>	<i>Hypocreales</i>	OSC 93610	AY184963	AY184974	EF469106	Sung et al. (2007)
<i>Cryphonectria parasitica</i>	<i>Diaporthales</i>	ATCC 38755	NG027589	DQ862048	DQ862017	Zhang et al. (2006)
<i>Cryptosporella hypodermiae</i>	<i>Diaporthales</i>	CBS 171.69	DQ862028	DQ862049	DQ862018	Zhang et al. (2006)
<i>Diaporthae eres</i>	<i>Diaporthales</i>	CBS 109767	AF408350	DQ471015	DQ470919	Spatafora et al. (2006)
<i>Diatrype disciformis</i>	<i>Xylariales</i>	CBS 197.49	DQ470964	DQ471012	DQ470915	Zhang et al. (2006)
<i>Eutypa lata</i>	<i>Xylariales</i>	CBS 208.87	DQ836903	DQ836896	DQ836889	Zhang et al. (2006)
<i>Falcocladium multivesiculatum</i>	<i>Falcocladales</i>	CBS 120386	JF831932	JF831928	–	Jones et al. (2014)
<i>Falcocladium sphaeropedunculatum</i>	<i>Falcocladales</i>	CBS 111292	JF831933	JF831929	–	Jones et al. (2014)
<i>Gelasinospora tetrasperma</i>	<i>Sordariales</i>	CBS 178.33	DQ470980	DQ471032	DQ470932	Spatafora et al. (2006)
<i>Glomerella cingulata</i>	<i>Glomerellales</i>	CBS 114054	AF543786	AF543762	DQ522441	Farr et al. (2006)
<i>Gnomonia gnomon</i>	<i>Diaporthales</i>	CBS 199.53	AF408361	DQ471019	DQ470922	Spatafora et al. (2006)
<i>Graphium penicillioides</i>	<i>Microascales</i>	CBS 506.86	AF027384	DQ471038	DQ470938	Spatafora et al. (2006)
<i>Halosphaeria appendiculata</i>	<i>Microascales</i>	CBS 197.60	U46885	U46872	–	Zhang et al. (2006)
<i>Hypocrea lutea</i>	<i>Hypocreales</i>	ATCC 208838	AF543791	AF543768	DQ522446	Spatafora et al. (2007)
<i>Kylindria peruamazonensis</i>	<i>Glomerellales</i>	CBS 838.91	GU180638	GU180609	GU180656	Réblová et al. (2011)
<i>Lasiosphaeria ovina</i>	<i>Sordariales</i>	CBS958.72	AY587946	AY083799	AY600286	Miller & Huhndorf (2004)
<i>Melanospora tiffanii</i>	<i>Melanosporales</i>	ATCC15515	AY015630	AY015619	AY015637	Zhang & Blackwell (2002)
<i>Melanospora zamiae</i>	<i>Melanosporales</i>	ATCC 12340	AY046579	AY046578	AY046580	Zhang & Blackwell (2002)
<i>Microascus trigonosporus</i>	<i>Microascales</i>	CBS 218.31	DQ470958	DQ471006	DQ470908	Spatafora et al. (2006)
<i>Monilochaetes infuscans</i>	<i>Glomerellales</i>	CBS 869.96	GU180639	GU180620	GU180657	O'Connell et al. (2012)
<i>Ophioceras dolichostomum</i>	<i>Magnaporthales</i>	CBS 114926	JX134689	JX134663	–	Luo & Zhang (2013)
<i>Ophioceras leptosporum</i>	<i>Magnaporthales</i>	CBS 894.70	JX134690	JX134664	–	Luo & Zhang (2013)
<i>Ophiocordyceps irangiensis</i>	<i>Hypocreales</i>	OSC 128578	DQ518770	DQ522556	DQ522445	Spatafora et al. (2007)
<i>Ophiodothella vaccinii</i>	<i>Phyllachorales</i>	ATCC 36333	–	U78777	–	Wanderlei-Silva et al. (2003)
<i>Ophiostoma piliferum</i>	<i>Ophiostomatales</i>	CBS 158.74	DQ470955	DQ471003	DQ470905	Spatafora et al. (2006)
<i>Ophiostoma stenoceras</i>	<i>Ophiostomatales</i>	CBS 139.51	DQ836904	DQ836897	DQ836891	Zhang et al. (2006)
<i>Papulosa amerospora</i>	<i>Cordanales</i>	JK 5547F	DQ470950	DQ470998	DQ470901	Spatafora et al. (2006)
<i>Polystigma amygdalinum</i>	<i>Phyllachorales</i>	EA-1	KM111540	KM111539	–	Habibi et al. (2015)
<i>Pseudohalonectria lignicola</i>	<i>Magnaporthales</i>	M95	JX134691	JX134665	–	Luo & Zhang (2013)
<i>Roumegueriella rufula</i>	<i>Hypocreales</i>	GJS 91-164	EF469082	EF469129	EF469116	Sung et al. (2007)
<i>Sordaria fimicola</i>	<i>Sordariales</i>	CBS 15.5973	AY545728	AY545724	AY780194	Zhang et al. (2006)
<i>Sordaria macrospora</i>	<i>Sordariales</i>	AFTOL-ID 393	AY346301	AY641007	AY641074	Huhndorf et al. (2004)
<i>Sphaerodothis acrocomiae</i>	<i>Phyllachorales</i>	–	–	U76340	–	Wanderlei-Silva et al. (2003)
<i>Sphaerostilbella berkeleyana</i>	<i>Hypocreales</i>	CBS 102308	U00756	AF543770	DQ522465	Spatafora et al. (2007)
<i>Togninia vibratilis</i>	<i>Togniniales</i>	CBS 117115	DQ649065	–	HQ878611	Réblová & Mostert (2007)
<i>Tolypocladium capitatum</i>	<i>Hypocreales</i>	OSC 71233	AY489721	AY489689	DQ522421	Spatafora et al. (2007)
<i>Tolypocladium japonicum</i>	<i>Hypocreales</i>	OSC 110991	DQ518761	DQ522547	DQ522428	Spatafora et al. (2007)
<i>Valsa ambiens</i>	<i>Diaporthales</i>	AR 3516	AF362564	DQ862056	DQ862025	Zhang et al. (2006)
<i>Verticillium dahliae</i>	<i>Glomerellales</i>	ATCC 16535	DQ470945	AY489705	DQ522468	Spatafora et al. (2006)
<i>Xylaria acuta</i>	<i>Xylariales</i>	ATCC 56487	AY544676	AY544719	DQ247797	Zhang et al. (2006)

this analysis was to infer the phylogenetic relationships within *Phyllachorales*. *Camarops ustulinoides* and *Camarops microspora* (*Boliniales*) were used as outgroup taxa, because some of our previous analyses (unpubl. data) have shown *Boliniales* as the sister group of *Phyllachorales*. The taxa of *Phyllachorales* used in both analyses are listed in Table 2 together with their location, host plant, and GenBank accession numbers. The alignments were deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S19724>).

PartitionFinder v. 1.1.1 (Lanfear et al. 2012) following Akaike Information Criterion (AIC) was used to select the best-fit model of evolution for each gene fragment separately for Bayesian and Maximum Likelihood (ML) analyses. Data were partitioned by gene and by codon position in the case of the protein-coding sequences. For the first dataset, a GTR+G model was applied to nrLSU, TrN+G model to nrSSU, and TIM+I+G to RPB2. For the second dataset, a TrNef+G model was applied

to ITS, TrN+G model to nrSSU, SYM+G model to *TEF1*, and TVMef+G to *RPB2*.

Phylogenetic tree inference

Phylogenetic analyses for each dataset were conducted applying Maximum Likelihood (ML) and Bayesian methods. The ML analyses were performed in RAXML (Stamatakis 2014) implemented in raxmlGUI v. 0.9b2 (Silvestro & Michalak 2012). One thousand non-parametric bootstrap iterations were used with the available models of generalized time reversible (GTR-GAMMA model) and a discrete gamma distribution (Stamatakis et al. 2008). Bayesian analyses were performed with the program MrBayes v. 3.2.6 (Ronquist et al. 2012) on XSEDE (Miller et al. 2010) in the CIPRES Science Gateway web portal ([http://www.phylo.org/sub\\_sections/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

Table 2 Taxa of *Phyllachorales* used in this study. Sequences in **bold** were isolated/sequenced in the present study.

Species	Locality	Voucher	Host	Host family	GenBank Accession Numbers				
					nrLSU	nrSSU	ITS	<i>RPB2</i>	<i>TEF1</i>
<i>Camarotella costaricensis</i>	Panama	MM-149	<i>Acrocomia aculeata</i>	<i>Arecaceae</i>	<b>KX430484</b>	<b>KX451863</b>	<b>KX451913</b>	<b>KX451954</b>	<b>KX451982</b>
	Panama	MM-21	<i>Acrocomia aculeata</i>	<i>Arecaceae</i>	<b>KX430490</b>	<b>KX451851</b>	<b>KX451900</b>	<b>KX451963</b>	<b>KX451988</b>
<i>Camarotella</i> sp.	Panama	MM-27	Unknown	<i>Arecaceae</i>	<b>KX430492</b>	<b>KX451852</b>	<b>KX451901</b>	–	–
<i>Coccodiella melastomatum</i>	Venezuela	CMU78543	<i>Miconia</i> sp.	<i>Melastomataceae</i>	–	U78543	–	–	–
<i>Coccodiella miconiae</i>	Panama	ppMP1342	<i>Miconia</i> sp.	<i>Melastomataceae</i>	<b>KX430506</b>	<b>KX451871</b>	–	–	–
<i>Coccodiella miconiicola</i>	Panama	TH571	<i>Ossaea micrantha</i>	<i>Melastomataceae</i>	<b>KX430512</b>	<b>KX451880</b>	–	–	–
<i>Coccodiella</i> sp.	Ecuador	MM-165	Unknown	<i>Melastomataceae</i>	<b>KX430488</b>	<b>KX451865</b>	<b>KX451917</b>	<b>KX451957</b>	<b>KX451986</b>
<i>Coccodiella toledo</i>	Venezuela	Unknown	<i>Miconia</i> sp.	<i>Melastomataceae</i>	–	U78544	–	–	–
<i>Coccolicola californica</i>	USA	F59034	<i>Washingtonia robusta</i>	<i>Arecaceae</i>	<b>KX430468</b>	<b>KX451866</b>	<b>KX451918</b>	<b>KX451958</b>	<b>KX451995</b>
	USA	F59038	<i>Washingtonia robusta</i>	<i>Arecaceae</i>	<b>KX430469</b>	<b>KX451867</b>	<b>KX451919</b>	<b>KX451959</b>	<b>KX451996</b>
<i>Phyllachora graminis</i>	Unknown	Unknown	Unknown	<i>Poaceae</i>	–	–	AF257111	–	–
	Canada	DAOM240981	Unknown	<i>Poaceae</i>	–	–	HQ317550	–	–
	Germany	RoKi3084	<i>Arrhenatherum elatius</i>	<i>Poaceae</i>	–	<b>KX451872</b>	–	–	–
	Germany	MM-166	<i>Hordelymus europaeus</i>	<i>Poaceae</i>	–	<b>KX451869</b>	<b>KX451920</b>	<b>KX451962</b>	<b>KX452001</b>
	Panama	TH544	<i>Dichantherium viscidellum</i>	<i>Poaceae</i>	<b>KX430508</b>	<b>KX451873</b>	–	–	–
	Sweden	UME31349	Unknown	<i>Poaceae</i>	–	–	AF064051	–	–
<i>Phyllachora maydis</i>	USA	BPI893231	<i>Zea mays</i>	<i>Poaceae</i>	–	–	KU184459	–	–
<i>Phyllachora</i> sp. 1	Thailand	MM-130	Unknown	<i>Poaceae</i>	–	<b>KX451883</b>	–	<b>KX451949</b>	<b>KX451976</b>
<i>Phyllachora</i> sp. 2	Thailand	MM-128	Bamboo	<i>Poaceae</i>	–	<b>KX451859</b>	<b>KX451908</b>	<b>KX451964</b>	<b>KX451973</b>
<i>Phyllachora</i> sp. 2	Thailand	MM-129	Bamboo	<i>Poaceae</i>	–	<b>KX451860</b>	<b>KX451909</b>	<b>KX451948</b>	<b>KX451974</b>
<i>Phyllachora</i> sp. 3	Costa Rica	MM-135	<i>Chusquea longifolia</i>	<i>Poaceae</i>	–	<b>KX451885</b>	–	<b>KX451951</b>	<b>KX451978</b>
<i>Phyllachora</i> sp. 3	Costa Rica	MM-78	<i>Chusquea</i> sp.	<i>Poaceae</i>	–	<b>KX451853</b>	–	<b>KX451942</b>	<b>KX451990</b>
<i>Phyllachora</i> sp. 3	Costa Rica	MM-98	<i>Chusquea longifolia</i>	<i>Poaceae</i>	<b>KX430502</b>	<b>KX451856</b>	–	<b>KX451945</b>	<b>KX451994</b>
<i>Phyllachora</i> sp. 3	Costa Rica	MM-134	<i>Chusquea longifolia</i>	<i>Poaceae</i>	<b>KX430479</b>	<b>KX451884</b>	–	<b>KX451968</b>	–
<i>Phyllachora</i> sp. 3	Ecuador	SO-07	<i>Chusquea</i> sp.	<i>Poaceae</i>	–	<b>KX451890</b>	–	–	<b>KX452009</b>
<i>Phyllachora</i> sp. 4	Benin	RMB1061	<i>Panicum maximum</i>	<i>Poaceae</i>	–	<b>KX451870</b>	<b>KX451921</b>	–	<b>KX452002</b>
<i>Polystigma pusillum</i>	Costa Rica	MM-113	<i>Andira inermis</i>	<i>Fabaceae</i>	<b>KX430474</b>	<b>KX451858</b>	<b>KX451907</b>	<b>KX451947</b>	<b>KX451972</b>
	Costa Rica	MM-147	<i>Andira inermis</i>	<i>Fabaceae</i>	–	<b>KX451862</b>	–	–	<b>KX451981</b>
	Panama	MM-19	<i>Andira inermis</i>	<i>Fabaceae</i>	<b>KX430489</b>	<b>KX451850</b>	<b>KX451899</b>	<b>KX451941</b>	<b>KX451987</b>
<i>Polystigma</i> sp.	Ecuador	MM-163	<i>Paspalum</i> sp.	<i>Poaceae</i>	<b>KX430487</b>	<b>KX451864</b>	<b>KX451916</b>	–	<b>KX451985</b>
<i>Serenomyces phoenicis</i>	USA	PLM314	<i>Phoenix canariensis</i>	<i>Arecaceae</i>	–	<b>KX451868</b>	<b>KX451928</b>	<b>KX451960</b>	<b>KX451997</b>
	USA	PLM315	<i>Phoenix canariensis</i>	<i>Arecaceae</i>	<b>KX430505</b>	<b>KX451886</b>	–	<b>KX451961</b>	<b>KX451998</b>
<i>Telimena aequatoriensis</i>	Ecuador	SO-05	<i>Monnina hirta</i>	<i>Polygalaceae</i>	–	<b>KX451889</b>	–	–	<b>KX452008</b>
<i>Telimena bicincta</i>	Costa Rica	MM-133	<i>Picramnia antidesma</i>	<i>Picramniaceae</i>	<b>KX430478</b>	<b>KX451861</b>	<b>KX451910</b>	<b>KX451950</b>	<b>KX451977</b>
	Costa Rica	MM-108	<i>Picramnia antidesma</i>	<i>Picramniaceae</i>	–	<b>KX451857</b>	<b>KX451906</b>	<b>KX451946</b>	<b>KX451971</b>
<i>Telimena canafistulae</i>	Panama	MM-13	<i>Cassia fistula</i>	<i>Fabaceae</i>	<b>KX430477</b>	<b>KX451849</b>	<b>KX451898</b>	–	<b>KX451975</b>
<i>Telimena engleri</i>	Ecuador	MM-153	<i>Anthurium</i> sp.	<i>Araceae</i>	–	<b>KX451888</b>	<b>KX451914</b>	<b>KX451955</b>	<b>KX451983</b>
	Ecuador	MM-159	<i>Anthurium</i> sp.	<i>Araceae</i>	–	–	<b>KX451915</b>	<b>KX451956</b>	<b>KX451984</b>
	Panama	TH551	<i>Anthurium concinnum</i>	<i>Araceae</i>	<b>KX430511</b>	<b>KX451875</b>	<b>KX451895</b>	<b>KX451939</b>	<b>KX451969</b>
	Ecuador	SO-09	<i>Anthurium</i> cf. <i>triphylum</i>	<i>Araceae</i>	–	–	<b>KX451934</b>	–	<b>KX452010</b>
<i>Telimena leaeae</i>	Panama	TH549	<i>Cissus trianae</i>	<i>Vitaceae</i>	<b>KX430509</b>	<b>KX451874</b>	–	–	–
<i>Telimena picramniae</i>	Panama	MM-05	<i>Picramnia</i> sp.	<i>Picramniaceae</i>	<b>KX430470</b>	<b>KX451848</b>	<b>KX451896</b>	<b>KX451940</b>	<b>KX451970</b>
<i>Telimena</i> sp. 1	Panama	MM-143	<i>Eugenia acapulcensis</i>	<i>Myrtaceae</i>	–	<b>KX451887</b>	<b>KX451911</b>	<b>KX451952</b>	<b>KX451979</b>
<i>Telimena</i> sp. 1	Panama	MM-144	<i>Eugenia acapulcensis</i>	<i>Myrtaceae</i>	–	–	<b>KX451912</b>	<b>KX451953</b>	<b>KX451980</b>
<i>Telimena</i> sp. 2	Costa Rica	MM-92	<i>Eugenia</i> sp.	<i>Myrtaceae</i>	<b>KX430501</b>	<b>KX451855</b>	<b>KX451905</b>	<b>KX451944</b>	<b>KX451993</b>
<i>Telimena</i> sp. 3	Costa Rica	MM-88	<i>Symplocos panamensis</i>	<i>Symplocaceae</i>	<b>KX430499</b>	<b>KX451854</b>	<b>KX451904</b>	<b>KX451943</b>	<b>KX451991</b>
<i>Telimena</i> sp. 4	Costa Rica	MM-47	<i>Rinorea</i> sp.	<i>Violaceae</i>	–	–	<b>KX451902</b>	–	<b>KX451989</b>
<i>Telimena</i> sp. 5	Ecuador	SO-14	<i>Wettinia</i> sp.	<i>Arecaceae</i>	–	<b>KX451892</b>	<b>KX451936</b>	–	–
<i>Telimena</i> sp. 5	Ecuador	SO-21	<i>Wettinia</i> sp.	<i>Arecaceae</i>	–	<b>KX451893</b>	<b>KX451937</b>	–	<b>KX452012</b>
<i>Telimena</i> sp. 5	Ecuador	SO-22	Unknown	<i>Arecaceae</i>	–	<b>KX451894</b>	<b>KX451938</b>	–	<b>KX452013</b>
<i>Telimena ulei</i>	Ecuador	SO-12	<i>Dioscorea meridensis</i>	<i>Dioscoreaceae</i>	–	<b>KX451891</b>	<b>KX451935</b>	–	<b>KX452011</b>
	Panama	TH574	<i>Dioscorea urophylla</i>	<i>Dioscoreaceae</i>	–	<b>KX451877</b>	–	–	–
<i>Telimena zanthoxylicola</i>	Panama	TH550	<i>Zanthoxylum scheryi</i>	<i>Rutaceae</i>	<b>KX430510</b>	<b>KX451879</b>	–	–	–



million generations, with trees sampled every 1 000th generation. Burn-ins were determined by checking the likelihood trace plots in Tracer v. 1.6 (Rambaut et al. 2014) and subsequently discarded. Tracer and the online version of AWTY (Nylander et al. 2008) were used to test convergence; no indication of lack of convergence was detected. Bayesian posterior probabilities (BPP)  $\geq 95\%$  and Bootstrap values (BS)  $\geq 70\%$  were considered to be significant.

#### *Ancestral state reconstruction of morphological and ecological characteristics*

An ancestral state reconstruction of four morphological and ecological characteristics used to delimit genera in *Phyllachorales* was performed with the Likelihood Ancestral States method of Mesquite v. 2.74 with an asymmetrical two-parameter model for binary data and a Mk1 model for multistate data (Maddison & Maddison 2015). The likelihood decision threshold value was set to 2. The Bayesian consensus tree based on the four-locus dataset was used for this reconstruction. The analysis was restricted to members of the *Phyllachorales*. The characteristics considered were: monocotyledonous or eudicotyledonous host plant, position of the perithecia in the leaves (completely immersed, erumpent, subcuticular, or superficial), the presence or absence of clypeus, and the colour of the stroma (black or brightly coloured). Other characteristics such as the family of the host plant, the presence or absence of ascospore septa, ascospore colour (hyaline or brown), and the anamorphic state also were considered, but they did not yield conclusive results because they lacked variation or data were missing for numerous taxa. Observations were taken from the respective specimen and from published literature. The outgroups were coded as uncertain. The character matrix used for this analysis is provided in Appendix 1.

## RESULTS

### *Sequences and alignments produced in this study*

We generated a total of 156 sequences from 27 species of *Phyllachorales*: 23 sequences of nrLSU, 40 of nrSSU, 31 of ITS, 26 of *RPB2*, and 36 of *TEF1*.

### *Congruence among loci*

For the three-locus dataset, CADM results showed no significant incongruence among loci, thus allowing concatenation of the three loci. The null hypothesis of complete incongruence among loci was rejected ( $W = 0.75$ ;  $p < 0.0001$ ). For the four-locus dataset, the null hypothesis of complete incongruence among loci was also rejected ( $W = 0.48$ ;  $p < 0.0001$ ), and the four loci were concatenated. Initially, we had planned to compile a five-locus dataset, also including nrLSU, however, the null hypothesis of complete incongruence among loci was accepted for nrLSU ( $W = 0.37$ ;  $p > 0.05$ ), and thus we did not consider nrLSU in the concatenated dataset.

### *Phyllachorales within Sordariomycetes*

The separately aligned datasets for each marker consisted of 76 sequences/790 base pairs for nrLSU, 77/908 for nrSSU, and 61/939 for *RPB2*. The three-locus dataset consisted of 88 specimens representing 72 species in 16 orders of *Sordariomycetes*. The final alignment was 2 637 base pairs in length. No conflicts were detected among the phylogenies produced by ML and Bayesian analyses; therefore we present only the ML tree for this dataset (Fig. 2). Support values for nodes were consistently higher in Bayesian analyses than in ML analyses. The phylogenies inferred from individual genes (data not shown) and the three-locus phylogeny (Fig. 2) showed the *Sordariomy-*

*cetes* as a robust monophyletic clade comprising three well-supported subclasses, *Hypocreomycetidae*, *Xylariomycetidae*, and *Sordariomycetidae*, with *Phyllachorales* grouping within *Sordariomycetidae*. The *Phyllachorales* appeared as a monophyletic, but moderately to weakly supported clade (0.94/77), including taxa of the two families *Phaeocharaceae* and *Phyllachoraceae* with the *Boliniales* as a sister group (0.97/74).

Three phyllachoraceous taxa fell outside the *Phyllachorales* clade. *Polystigma amygdalinum* and *Ophiodothella vaccinii* were located with weak support within the *Xylariomycetidae*. The single sequence representing *Sphaerodothis acrocomiae* formed a weakly supported clade together with taxa of *Hypocreales* (Fig. 2). This sequence may stem from a hypocrealen hyperparasite. It was not possible, however, to test possible incongruence regarding these taxa since they are represented by sequences downloaded from GenBank and two of them were represented by only one of the markers (nrSSU).

### *Phylogenetic relationships within the Phyllachorales*

The four-locus dataset included 53 sequences representing 29 species of *Phyllachorales*. The dataset was supplemented with additional sequences from GenBank (two SSU and four ITS sequences). The alignment consisted of 2 728 total characters.

The topology of the tree identified by Bayesian analysis was almost identical to the one obtained by the ML analyses, therefore we present the Bayesian tree for this dataset (Fig. 3). In the Bayesian tree, the 51 sequences of *Phyllachorales* clustered into one major clade with high support (100/1.0). Within the *Phyllachorales*, three clades (I–III) can be identified.

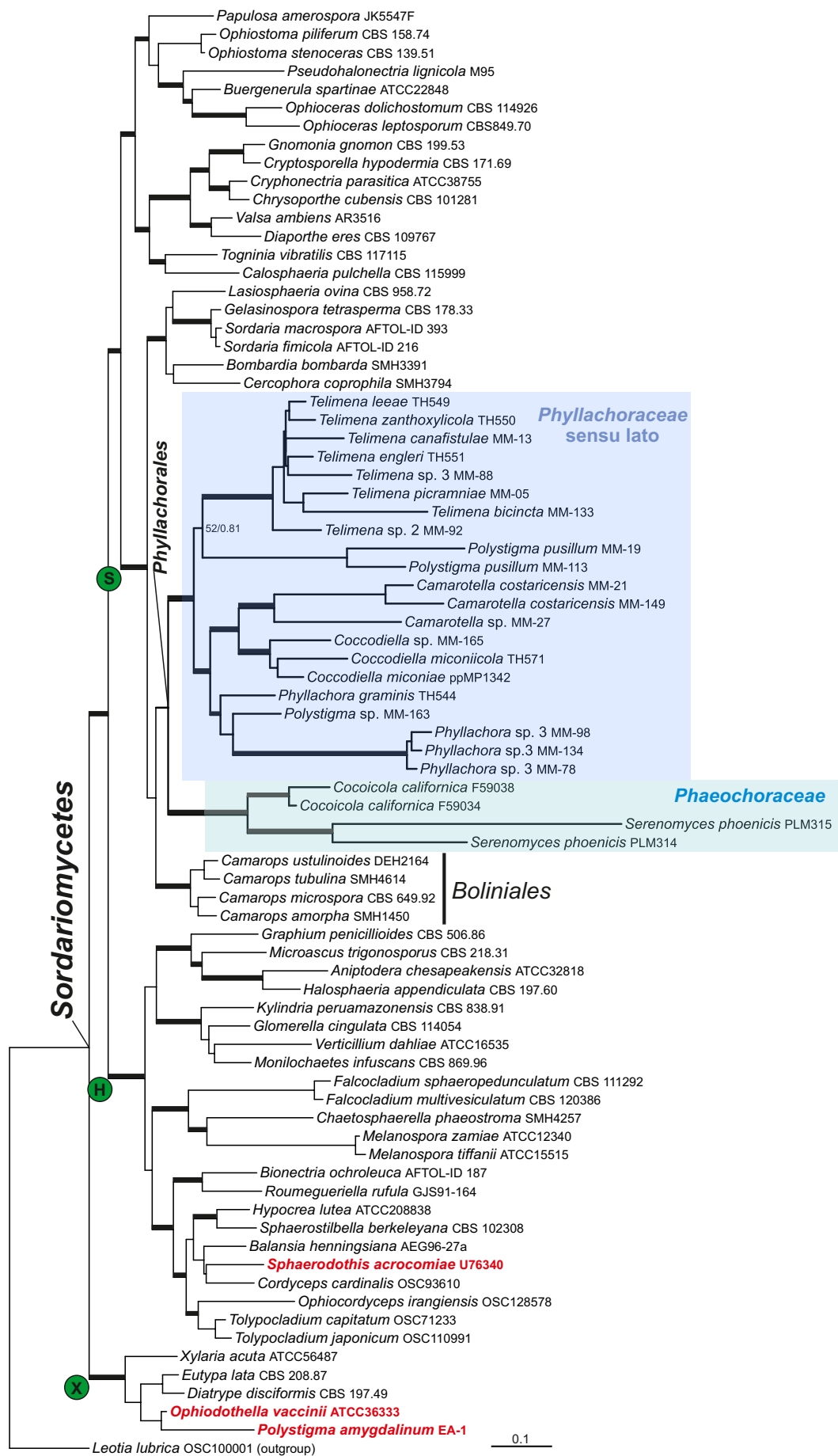
Clade I, which received weak support in the ML analysis (0.98/55), was divided into three subclades: the well-supported subclade one containing members of the genera *Camarotella* on *Arecaceae* and *Coccodiella* on *Melastomataceae*; subclade two containing the type species *Phyllachora graminis*, other species of *Phyllachora*, and one species of *Polystigma*, all of them growing on *Poaceae*; and subclade three including other graminicolous species of *Phyllachora* on *Chusquea* spp. and *Polystigma pusillum* on *Fabaceae*.

Clade II (1.0/100) is a monophyletic, strongly supported group restricted to species growing on *Arecaceae*, i.e., members of the genera *Cocoicola* and *Serenomyces* in the family *Phaeocharaceae*.

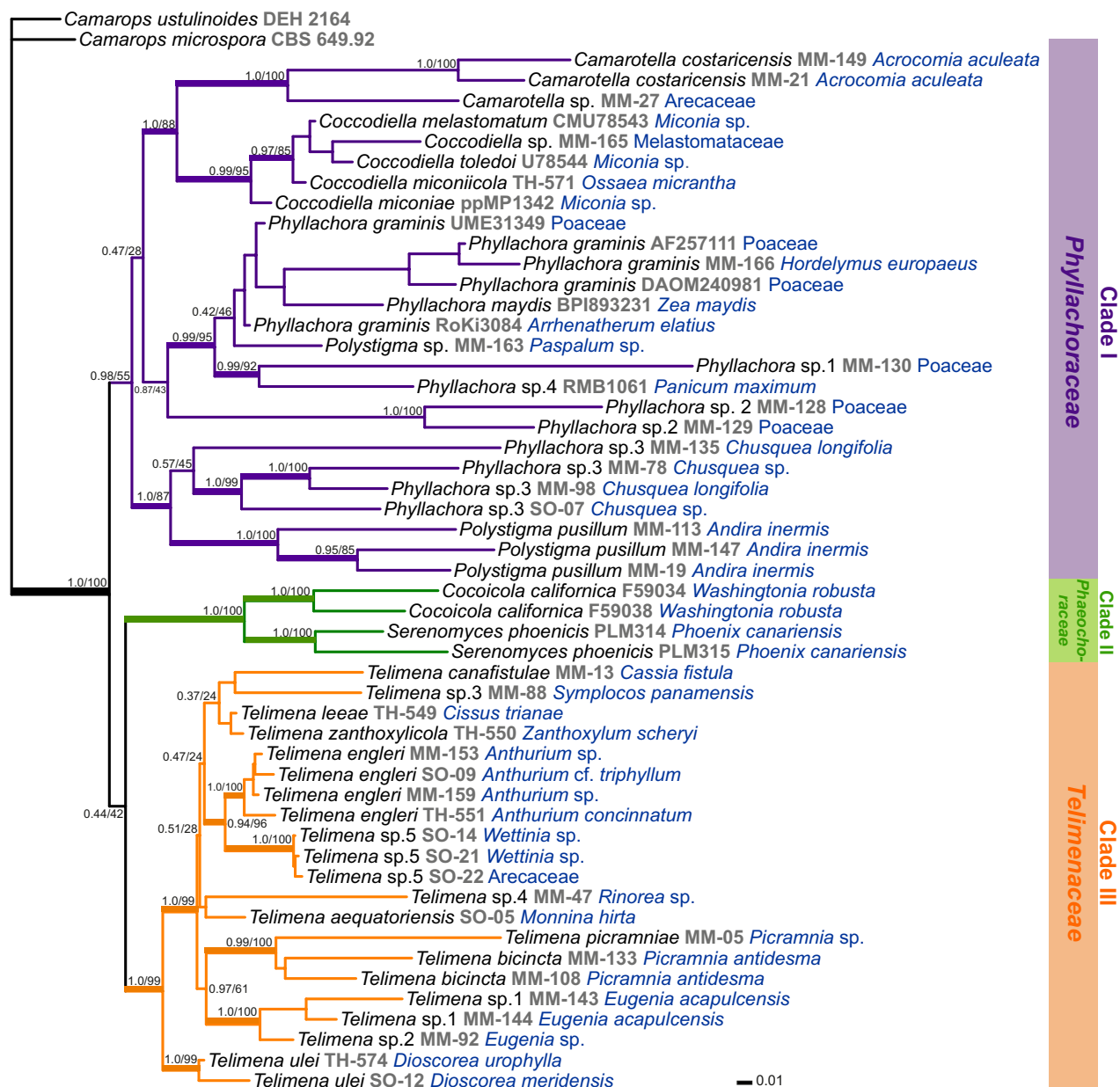
Clade III is also strongly supported (1.0/99) and included species of tar spot fungi with immersed perithecia and infecting species belonging to many different plant families, but not *Poaceae*. Two subclades can be distinguished, one containing members growing on several monocotyledonous and eudicotyledonous host families and a second group with species growing on *Dioscoreaceae*. The internal relationships within Clade III were mostly unresolved, with low posterior probability and bootstrap values. Clades II and III may be sister groups but this relationship was not strongly supported (0.44/42).

Our results indicated that the family *Phyllachoraceae* and the genus *Phyllachora* are polyphyletic being represented in two distinct clades, called Clades I and III here. The genus *Polystigma* is polyphyletic, with the three species treated in this study included in three groups, one in *Xylariomycetidae* and the other two within two different lineages within Clade I.

There were several examples of different species from the same host species, genus, or family forming supported clades, for example two different species growing on *Picramnia* spp. (*Picramniaceae*), several species on *Eugenia* spp. (*Myrtaceae*) or *Chusquea* spp. (*Poaceae*), and *Coccodiella* spp. on *Melastomataceae*.



**Fig. 2** Phylogenetic position of *Phyllachorales* within *Sordariomycetes*. This is a Maximum Likelihood phylogeny based on three nuclear markers (nrLSU, nrSSU, *RPB2*). Support values are ML bootstrap values based on 1 000 replicates, and posterior probabilities from a Bayesian analysis. Nodes receiving ML bp > 70 %, or Bayesian PP > 0.94 are considered as strongly supported and are indicated by thickened branches; see TreeBASE files for individual support. Phyllachoraceous taxa which fall outside the order are indicated in red. Abbreviations: S = subclass *Sordariomycetidae*; H = subclass *Hypocreomycetidae*; X = subclass *Xylariomycetidae*.



**Fig. 3** Phylogenetic relationships within the order *Phyllachorales*. This is a Bayesian analyses based on four nuclear markers (nrSSU, ITS, *RPB2*, *TEF1*). Support values are posterior probabilities from a Bayesian analysis and ML bootstrap values based on 1 000 replicates. Nodes receiving Bayesian PP > 0.94, or ML bp > 70 % are considered as strongly supported and are indicated by thickened branches. Hosts are indicated in blue text.

### Ancestral state reconstruction of ecological and morphological characteristics

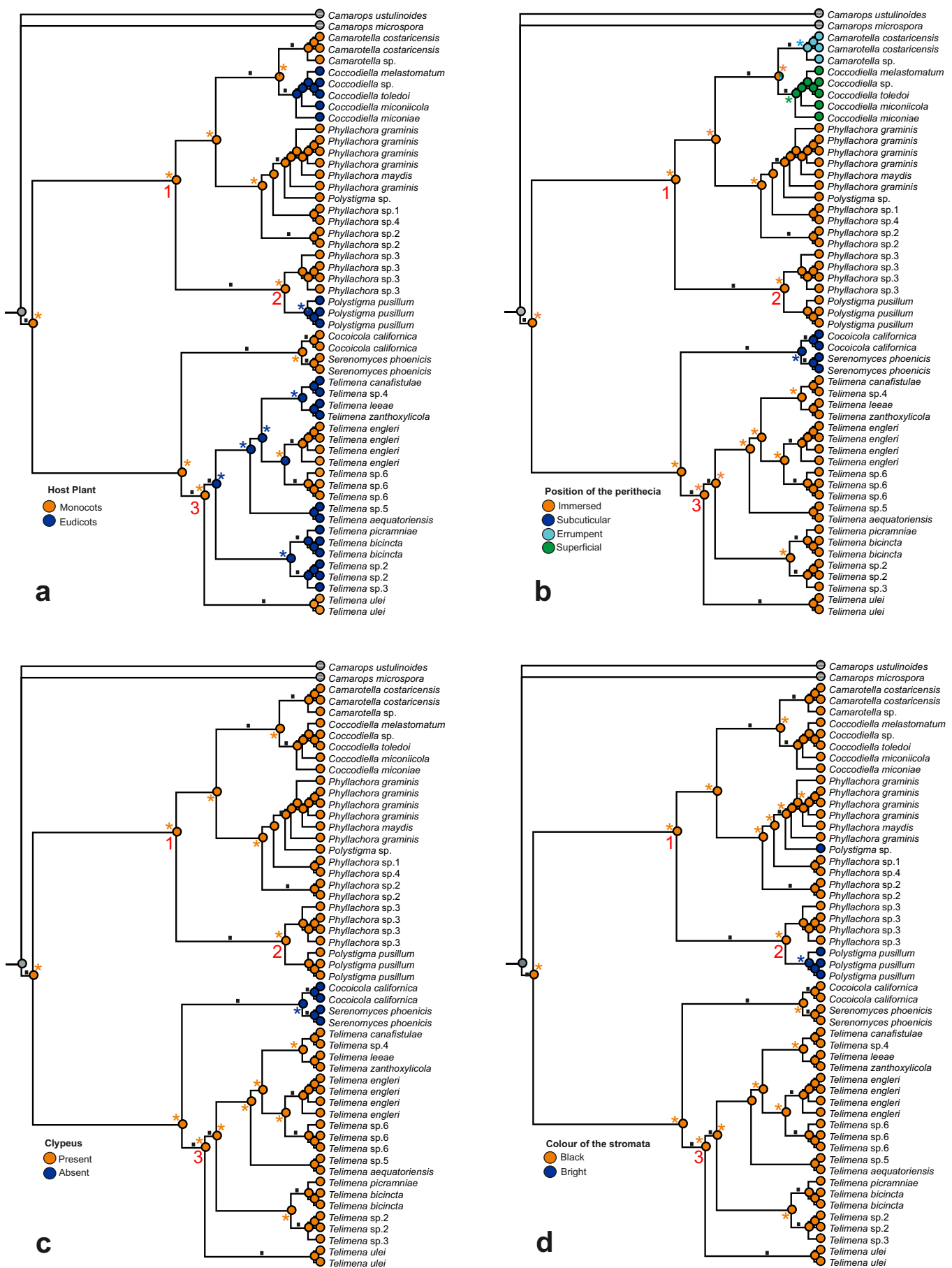
The evolution of one ecological and three morphological characteristics was reconstructed by employing the Bayesian tree sampling of the four-locus dataset (Fig. 4a–d). The analysis of the host relationships suggested that the ancestor of *Phyllachorales* was growing most likely on a monocotyledonous plant (Proportional Likelihood (PL) 0.9966, Fig. 4a). The ancestor of each clade most probably was also growing on a monocotyledonous plant (Clade I, PL = 0.9981; Clade II, PL = 0.9960; Clade III, PL = 0.9685). The position of the perithecia in the leaves varies from immersed in the mesophyll to superficial (Fig. 1a–f). This reconstruction suggested that for species of *Phyllachorales* the ancestral state were perithecia completely immersed in the mesophyll (PL = 0.9988, Fig. 4b), while erumpent or superficial perithecia apparently evolved at least once each within Clade I. The presence of subcuticular perithecia was supported as the ancestral state in Clade II (PL = 0.9928). The ancestor of *Phyllachorales* was predicted

to have had a clypeus (PL = 0.9989) while the lack of a clypeus was predicted as the ancestral state (PL = 0.9933) for species within Clade II (Fig. 4c). A black colour of stromata was well supported as the ancestral state (PL = 0.9999) for *Phyllachorales*, and bright coloured stromata apparently evolved at least twice in Clade I (Fig. 4d).

### TAXONOMY

Based on the phylogenetic relationships revealed by this study, as well as the ecological and morphological characteristics of the species grouped in the observed clades within the *Phyllachorales*, three families were recognised: the *Phaeocho-raceae* were accepted as previously described (Hyde et al. 1997), the *Phyllachoraceae* and *Phyllachora* need to be emended, while the *Telimenaceae* are newly described here. The genus *Telimenia* is emended to accommodate established species of *Phyllachora* that belong to the family *Telimenaceae*.





**Fig. 4** Ancestral character state reconstruction in the *Phyllachorales* based on the Bayesian tree. All analyses are based on maximum likelihood reconstruction with asymmetrical two-parameter (a, c, d) or Mk1 (b) models. The characteristics considered were: a. Host plant (possible states are monocotyledonous or eudicotyledonous host plant); b. position of the perithecia in the leaves (immersed, erumpent, subcuticular, or superficial); c. presence or absence of clypeus; d. colour of the stroma (black or brightly coloured). Relative likelihood probabilities for each character state are represented with a pie chart at the nodes. Squares denote supported nodes for which posterior probabilities and bootstrap values are presented in Fig. 3. Coloured asterisks near pies indicate that the corresponding state is judged best according to the threshold.

**Phaeochoraceae** K.D. Hyde *et al.*

Species of *Phaeochoraceae* present black stromata, usually significantly raising the substratum surface, perithecia immersed, clustered forming a single cavity, embedded in pseudo-stromata and not covered by a clypeus as in other species of *Phyllachorales*. Ascospores are typically thick-walled, olivaceous to brownish, aseptate and usually with a delicate striate ornamentation. They are biotrophic or saprotrophic on palms. For a more detailed description of this family see Hyde *et al.* (1997).

Genera included in this family: *Cocoicola*, *Phaeochora*, *Phaeochoropsis*, *Serenomyces*.

**Phyllachoraceae** Theiss. & P. Syd., Ann. Mycol. 13, 3/4: 168. 1915. emend. Mardones, Trampe & M. Piepenbr.

*Stroma* of various shapes, covered by a cuticular or epidermal shiny black clypeus, sometimes bright coloured, development around the ostioles of perithecia. *Ascomata* perithecioid, amphigenous, epiphyllous or hyphophyllous, uni- to multiloculate, sometimes confluent, frequently surrounded by a bright yellow to reddish discolouration zone, and when superficial with an hypostroma anchoring the ascomata with the host tissue. *Pseudostroma* sparse or absent. *Perithecia* superficial, erumpent or immersed in the host tissue, pyriform, globose, lenticular, or deformed by vascular bundles, with a periphysate ostiole, with a hyaline to pigmented peridium composed of *textura intricata*. *Paraphyses* hyaline, thin-walled, slightly longer than the asci, septate, often dissolving during maturation. *Asci* unitunicate, clavate or cylindrical, usually 8-spored, rarely 4-spored, apical ring normally not turning blue in iodine reagent (J), sometimes with an ascus crown. *Ascospores* usually hyaline, rarely pale brown, globose to filiform, mostly cylindrical, thin and smooth-walled, mostly aseptate, sometimes surrounded by gel. Structures supposed to be *spermogonia* infrequently found, pycnidial, spermatogenous cells cylindrical, tapering towards the tip, proliferating percurrently, producing filiform, hyaline, aseptate scolecospores that are probably spermatial in function.

Mostly biotrophic, growing mainly on members of *Poaceae*, but also associated with other families.

*Generic type of the family.* *Phyllachora* Nitschke ex Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 216. 1870 (1869–1870).

**Phyllachora** Nitschke ex Fuckel., Jahrb. Nassauischen Vereins Naturk. 23–24: 216 (1870) emend. Mardones, Trampe & M. Piepenbr.

*Etymology.* Name probably referring to the leaf habitat (*gr.* phyllas: leaf, chora: location, position).

*Type species.* *Phyllachora graminis* (Pers.) Nitschke. In Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 216. 1870 (1869–1870).

Infection spot variable in outline, often roundish, black, shiny. *Clypeus* mostly epidermal. *Pseudostroma* absent or sparse. *Perithecia* immersed in the host tissue. *Periphyses* present. *Paraphyses* filiform, septate, hyaline, often deliquescent. *Asci* cylindrical to clavate, with or without apical ring that does not stain blue in iodine, mostly 8-spored. *Ascospores* mostly hyaline, aseptate, smooth, mostly without gelatinous sheaths. *Spermogonia* acervulate or pycnidial, variable in shape, often associated with ascomata. *Spermatogenous cells* cylindrical, tapering toward the apex, proliferation percurrent. *Spermatia* filiform, curved, hyaline.

**Telimenaceae** Mardones, Trampe & M. Piepenbr., *fam. nov.* — MycoBank MB818222

*Stroma* of various shapes, covered by a cuticular or epidermal shiny blackened clypeus, which may have limited development around the ostiole or extensively above the ascomata and in some cases below the ascomata. *Ascomata* perithecioid, amphigenous, epiphyllous or hyphophyllous, uni- to multiloculate, sometimes confluent, frequently surrounded by a bright yellow to reddish discolouration zone. *Pseudostroma* strongly developed, interfusing and conspicuously expanding into the host tissue. *Perithecia* subcuticular, epidermal, subepidermal or immersed in the host tissue, pyriform, globose, lenticular, or deformed by vascular bundles, with a periphysate ostiole, with a hyaline to pigmented peridium composed of *textura intricata*. *Paraphyses* hyaline, thin-walled, slightly longer than the asci, septate, often dissolving during maturation. *Asci* unitunicate, clavate or cylindrical, usually 8-spored, rarely 4-spored, apical ring normally not turning blue in iodine reagent (J). *Ascospores* usually hyaline, rarely pale brown, globose to filiform, mostly cylindrical, thin and smooth-walled, aseptate to 3-septate, sometimes surrounded by gel. *Spermogonia* infrequently found, pycnidial, spermatogenous cells cylindrical, tapering towards the tip, proliferating percurrently, developing filiform, hyaline, aseptate scolecospores, probably spermatial in function.

Mostly biotrophic, growing on several monocotyledonous and dicotyledonous families, except *Poaceae*.

*Type genus.* *Telimenia* Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 18. 1900.

**Telimenia** Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 18. 1900. emend. Mardones, Trampe & M. Piepenbr.

*Etymology.* The name of the genus refers to the name of a Polish hero in literary works of A. Mickiewicz.

*Type species.* *Telimenia erythrinae* Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 18. 1900. JAVA, Merapi, on *Erythrina variegata* L. (as *E. lithosperma* Miq.), s.d., Raciborski s.n. (type IMI302320!).

Infection spots dark, on living or dead leaves. *Ascomata* solitary to aggregated, subcuticular, epidermal, subepidermal or immersed in the host tissue, amphigenous, epiphyllous or hyphophyllous, clypeate, uni- to multilocular, ostiolate. *Clypeus* subcuticular or epidermal. *Pseudostroma* strongly developed. *Hama-thecium* with periphyses in the ostiole and evanescent paraphyses. *Asci* unitunicate, cylindrical to broadly ellipsoidal, with iodine-negative apical ring, 8-spored. *Ascospores* hyaline to pale brown when mature, globose to filiform, straight to curved, smooth, 0–3-septate.

*Additional specimens examined.* **Telimenia bicincta**. COSTA RICA, San Jose, on *Picramnia antidesma*, 10 Mar. 1890, A. Tonduz 2183 (type BR-76016-65). **Telimenia ecastophylli** (as '*T. caudata*'). ECUADOR, on *Pterocarpus amazonum* (as *P. ulei*), 24 Feb. 1938, H. Sydow s.n. (IMI307885); on *Pterocarpus* sp., 22 Feb. 1938, H. Sydow s.n. (IMI346458). VENEZUELA, Puerto La Cruz, El Limón, *Pterocarpus rohrii*, 22 Jan. 1928, H. Sydow s.n. (IMI18828). **Telimenia graminella**. PHILIPPINES, Luzon, on *Paspalum* sp., Sept. 1913, M. Ramos in *Flora of the Philippines* 8224 (type IMI18829). **Telimenia haraeana** (as '*T. arundinariae*'). JAPAN, Nagato Prov., Shimonoseki, on *Pleio-blastus simonii*, 5 May 1955, Katumoto s.n. (IMI63381). **Telimenia rhoinea** (as '*Homostegia rhoinea*'). USA, California, San Diego, on *Rhus integrifolia*, Mar. 1895, K. Brandegee No. 15 (type NY00830409).

Notes — The genus *Telimenia* was originally described by Raciborski (1900) for phyllachora-like species with 3-septate ascospores. Currently, 14 species have been described within this genus. Its type species, *T. erythrinae*, is a parasite of the dicotyledonous plant host *Erythrina variegata* (*Fabaceae*). In the past, *Telimenia* have been related with genera *Telimenopsis* (currently a synonym), *Telimenella* and *Telimenochora* (Petrak

1931, Müller 1975, Barr 1977). Morphological characteristics of the ascospores, i.e., shape, number of septa and position of the septa, have been used to separate these genera from each other. For a detailed description of the former and their main differences see Sivanesan (1987).

Examination of specimens of *Telimena* spp. (cited above, including type material) showed that stromata of *Telimena* spp. are similar to those of *Phyllachora* spp. with aseptate ascospores. Photos of sections of ascomata of *Ph. graminis* and *T. bicincta* are provided for comparison (Fig 1a, f). These sections show immersed perithecia in the mesophyll of the leaf, surrounded by pseudostroma. The stromatic development in species of *Telimena* seems rather variable, like in *Phyllachora* spp., with reduced stromatic development in species occurring in grasses and abundant pseudostroma in the remaining species. Our observations show that ascospores of *Phyllachora* spp. sometimes are septate when mature, and we repeatedly observed aseptate ascospores as well as ascospores with one, two or three septa in the same specimen.

The following taxa are combined into *Telimena* based on their phylogenetic position as shown by data presented herein. In addition, we propose a recently collected specimen of *Telimena bicincta* as epitype.

***Telimena aequatoriensis*** (Theiss. & Syd.) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818223

*Basionym.* *Phyllachora aequatoriensis* Theiss. & Syd., Ann. Mycol. 13, 5/6: 521. 1915.

*Synonym.* *Phyllachora dendritica* Rehm, Hedwigia 31: 305. 1892. ECUADOR, Quito, Río Machangara, on *Monnina* sp., 10 Apr. 1892, G. V. Lagerheim in Rehm 1072 Ex. Herb. Sydow (type S F9301).

***Telimena bicincta*** (E. Bommer & M. Rousseau) Theiss. & Syd., Ann. Mycol. 13, 5/6: 601. 1915

*Basionym.* *Montagnella bicincta* E. Bommer & M. Rousseau, Bull. Soc. Roy. Bot. Belgique 35: 163. 1896. COSTA RICA, San Jose, on *Picramnia antidesma*, 10 Mar. 1890, A. Tonduz 2183 (holotype BR-76016-65!).

*Epitype* (MycoBank MBT375061, designated here): COSTA RICA, San José, San Pedro Montes de Oca, Campus Universidad de Costa Rica, N9°56'17" W84°2'59", on *Picramnia antidesma*, 19 Jan. 2015, Mardones MM-133 (epitype USJ 108929; isoeotype M).

***Telimena canafistulae*** (F. Stevens & Dalbey) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818224

*Basionym.* *Phyllachora canafistulae* F. Stevens & Dalbey, Bot. Gaz. 68: 55. 1919. PUERTO RICO, Mayaguez, on *Cassia fistula*, 14 June 1915, F.L. Stevens 7022 (holotype ILL00011456!; isotypes BPI 636604, BPI 636617, BPI 844649!; K, MAPR, NY 00986162; fide Cannon 1991).

*Synonym.* *Phyllachora azuarenensis* Petr. & Cif., Ann. Mycol. 30, 3/4: 235. 1932. DOMINICAN REPUBLIC, Azua, on *Barbieria pinnata*, 25 Ago. 1929, E.L. Ekman 3565 in Herb. Ciferri (holotype BPI 636400 n.v.; isotypes NY 00986150, S F49803 n.v.).

***Telimena engleri*** (Speg.) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818225

*Basionym.* *Phyllachora engleri* Speg., Anales Soc. Ci. Argent. 19, 2: 96. 1885. PARAGUAY, Barrancas de San Antonio, on *Spathicarpa lanceolata*, Jan. 1882, B. Balansa 3746 (holotype LPS130!).

*Synonyms.* *Botryosphaeria anthuriicola* Massee, Bull. Misc. Inform. Kew: 185. 1899. COSTA RICA, Cartago, on *Anthurium gracile*, s.d., Donnell Smith 6813 (type K(M) 100501 n.v.; isotype BPI 797076 n.v.).

*Dothidella bifrons* Starbäck, Bih. Kongl. Svenska Vetensk.-Akad. Handl., Afd. 3 25, no. 1: 46. 1899. PARAGUAY, Concepcion, on *Araceae*, 17 Sept. 1893, G.A. Malme s.n. (holotype S F9201 n.v.).

*Phyllachora anthurii* (E. Bommer & M. Rousseau) Speg., Bol. Acad. Nac. Ci. Córdoba 23, 3-4: 567. 1919. (1918).

*Dothidea anthurii* E. Bommer & M. Rousseau, Bull. Soc. Roy. Bot. Belgique 35: 163. 1896.

*Phyllachora dioscoreae* Rehm, Hedwigia 36, 6: 370. 1897. BRAZIL, Brasilien, on leaves of *Dioscoreaceae*, s.d., E. Ule 217 in Herb. Berol. Ex Herb. Rehm (syntype S F218511 n.v.).

*Phyllachora engleri* f. *anthurii* Speg., Anales Soc. Ci. Argent. 26, 1: 37. 1888. PARAGUAY, on *Anthurium* sp., Sept. 1883, B. Balansa 4082-4106 (type LPS 130!).

*Phyllachora engleri* var. *anthurii* (Speg.) Pat., Bull. Herb. Boissier 3: 71. 1895.

*Phyllachora philodendri* Pat. (as '*philodendronis*'), Bull. Soc. Mycol. France 8, 3: 134. 1892. ECUADOR, on *Philodendron* sp., Jan. 1892, Lagerheim s.n. (type FH n.v.).

*Phyllachora phylloplaca* Chardón, Mycologia 32, 2: 197. 1940. BRAZIL, Vicosá, on *Diclidanthera laurifolia*, 22 Apr. 1933, Muller 491 (type CUP-MG-000491 n.v.).

*Additional specimens examined.* ***Dothidea phylloplaca*** (as '*phylloplacus*'). GUYANA, prope Cayennam, on unidentified leaves, s.d., Leprieur 1152 (type PC 96772!). ***Phyllachora ipirangae***. BRAZIL, Sao Paulo, Ipiranga, Villa Marianna, on *Eugenia* sp., 23 Aug. 1906, A. Usteri s.n. (type S F8918!). ***Sphaeria phylloplaca*** (as '*phylloplacus*'). SURINAM, on unidentified leaves, 1827, Weigelt s.n. (isotypes HBG 6597, PC 96768).

Notes — Currently, *P. engleri* is treated as a synonym of *Phyllachora phylloplaca* in Index Fungorum. Montagne (1855) published *Dothidea phylloplaca* and gave *Sphaeria phylloplaca* as synonym. Later, Saccardo (1883) cited Montagne's description of *D. phylloplaca* recombining the species to *P. phylloplaca*. Theissen & Sydow (1915) cited *Sphaeria phylloplaca* and *P. ipirangae*, as synonyms of *P. phylloplaca* on *Eugenia* sp. (*Myrtaceae*), and considered as distinct from *P. engleri*. However, this species is currently accepted as illegitimate (superfluous name). Re-examination of the type material of *D. phylloplaca* and *S. phylloplaca* confirmed that both specimens are growing on dicotyledonous plant hosts. Furthermore, *D. phylloplaca* is not accepted as a synonym of *S. phylloplaca* as described by Montagne (1855), because leaf material differs significantly in habitus and texture.

***Telimena leeeae*** (Koord.) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818226

*Basionym.* *Phyllachora leeeae* Koord., Verh. Kon. Akad. Wetensch., Afd. Natuurk., sect. 2, 13, 4: 182. 1907. JAVA, Gombong, on *Leea rubra*, 18 Mar. 1905, Kooders s.n. (holotype S F49896!).

***Telimena picramniae*** (Syd. & P. Syd.) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818227

*Basionym.* *Dothidella picramniae* Syd. & P. Syd., Ann. Mycol. 11, 3: 266. 1913. COSTA RICA, San Jose, on *Picramnia bonplandiana* (as *P. antidesma*), 10 Nov. 1912, Ad. Tonduz s.n. (isotype CUP Syd. F.exot.ex.0134 n.v.).

*Synonyms.* *Endodothella picramniae* (Syd. & P. Syd.) Syd., in Theissen & Sydow, Ann. Mycol. 13, 5/6: 590. 1915.

*Phyllachora picramniae* (Syd. & P. Syd.) Petr., Ann. Mycol. 38, 2/4: 259. 1940. *Phyllachora picramniae* F. Stevens, Illinois Biol. Monogr. (Urbana) 11, 2: 190. 1927. COSTA RICA, Aserri, on *Picramnia bonplandiana* (as *P. antidesma*), 26 June 1923, F.L. Stevens 119 (holotype ILL00005686!; isotypes BPI 639002!, CUP-014698, MICH 14837; paratypes CUP-014697, CUP-014699, ILL00005686, ILL00005687).

***Telimena ulei*** (G. Winter) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818228

*Basionym.* *Phyllachora ulei* G. Winter, Grevillea 15, no. 75: 90. 1887. BRAZIL, Sao Francisco, on unknown plant, Aug. 1884, Ule 143 (holotype S F8887!).

***Telimena zanthoxylicola*** (Seaver) Mardones, Trampe & M. Piepenbr., *comb. nov.* — MycoBank MB818229

*Basionym.* *Phyllachora zanthoxylicola* Seaver, Mycologia 20, 4: 225. 1928. JAMAICA, on *Zanthoxylum insularis*, s.d., E.G. Britton 443 (holotype NY 01089448!).



*Synonym. Telimenopsis fagarae* Speer, Trans. Brit. Mycol. Soc. 75, 3: 504. 1981 (1980). ECUADOR, Galapagos Islands, Insula Santa Cruz, on *Zanthoxylum fagara*, 16 Oct. 1976, *Gard & For. Nobis*, s.n. (holotype IMI 245878 n.v., fide Speer 1980).

## DISCUSSION

### *Phyllachorales* within *Sordariomycetes*

Our findings support the placement of *Phyllachorales* within the subclass *Sordariomycetidae* in the class *Sordariomycetes*, as suggested by previous molecular studies (Winka & Eriksson 2000, Wanderlei-Silva et al. 2003). The extended taxon sampling and the use of three markers (nrLSU, nrSSU, and *RPB2*) allow us to strongly corroborate these findings.

This study confirms that the *Phyllachorales* and *Boliniales* are closely related orders, disproving that the sister group of *Phyllachorales* may be the *Diaporthales* (Cannon 1988). Although the order *Boliniales* mostly comprises saprotrophic fungi, species of both orders have perithecia immersed in stromata and unitunicate asci with an inamyloid apical ring (Untereiner et al. 2013). Also, in some species of *Boliniales*, the black stromata are described as clypeate (Sivanesan 1975, Réblová 1997).

The *Phyllachorales* are supported as monophyletic based on the three-gene tree with moderate support. This is the first analysis that demonstrates the monophyly of the order including members of both families currently accepted in the order. The reason for the lack of a strong support for the monophyly of the order seems to be the sister group relationship among the three major clades. The three-locus dataset showed a close relationship between Clades I and III, and a separate Clade II while the four-locus dataset showed Clade II to be more closely related to Clade III. Species excluded from the *Phyllachorales* are *Polystigma amygdalinum*, *Ophiodothella vaccinii*, and *Sphaerodothis acrocomiae*. The reasons for the previous inclusion of these species in *Phyllachorales* were their biotrophic condition, immersed perithecia, and presence of stromatic tissue. Other molecular studies also supported these exclusions (Wanderlei-Silva et al. 2003, Habibi et al. 2015).

### *Phylogenetic relationships within the Phyllachorales: clade-based assessment*

Our current study presents the up to now largest analysis of the *Phyllachorales*, with four gene regions from 29 species, yielding the most reliable phylogenetic analyses of *Phyllachorales* so far. Based on this analysis, three distinct monophyletic clades can be distinguished within the *Phyllachorales*.

Clade I includes the type species of the order, *P. graminis*, together with other tar spot fungi on *Poaceae* having immersed stromata, *Po. pusillum* on *Fabaceae*, *Camarotella* spp. on palms, and *Coccodiella* spp. on *Melastomataceae*. Species on *Poaceae* are grouped in two subclades, one containing *P. graminis*, *P. maydis*, one species of *Polystigma* sp., and two species on bamboo from Thailand. The other subclade contains *Phyllachora* spp. growing on *Chusquea* spp. In our study, the ML analysis provided no support for this clade. However, due to the limited taxon sampling included in this phylogeny, it seems too early to further subdivide this clade. A better-sampled phylogenetic study of these species as well as more species on *Chusquea* spp., and other grasses are needed to resolve the systematics of these species. Within the clade, sequences of *P. graminis* show a high level of variation, so apparently *P. graminis* is an assemblage of cryptic species. The position of *Po. pusillum* remains uncertain. In the three-locus dataset, sequences of *Po. pusillum* formed a separate clade outside Clade III without support (0.81/52). The same occurred with the analysis restricted to the nrLSU marker. This situation was

the main cause of incongruent results that did not allow us to concatenate the five-locus dataset.

The other subclade in Clade I comprises *Camarotella* spp. with strongly erumpent and flattened stromata and *Coccodiella* spp. with superficial stromata. These two genera seem to be monophyletic and closely related. Hyde & Cannon (1999) suggested that the species of *Oxodeora* and *Coccostromopsis*, also with typically erumpent stromata, are probably closely related to *Coccodiella* and *Camarotella*. However, no molecular data is available so far to corroborate this relationship. These results indicate that, surprisingly, tar spot fungi on *Poaceae* with immersed perithecia are more closely related to *Camarotella* spp. and *Coccodiella* spp. with perithecia in erumpent stromata, than to species with rather similar immersed perithecia growing on other monocotyledonous and dicotyledonous host plants in Clade III.

*Polystigma* spp., which are leaf parasites with brightly coloured stromata, also are polyphyletic in Clade I (Cannon 1991). Three species of *Polystigma* were included in our analyses: *Po. amygdalinum* on *Prunus dulcis*, *Po. pusillum* on *Andira inermis* and *Polystigma* sp. on *Paspalum* sp. Several authors already suggested that the genus *Polystigma* is polymorphic, containing at least five well-defined assemblages of species (Cannon 1996, 1997, Pearce & Hyde 2006, Habibi et al. 2015). According to Cannon (1996) and his examination of the type species *Polystigma rubrus* on *Prunus domestica*, members of *Polystigma* should be restricted to species growing on Euro-Asiatic species of *Rosaceae* (*Prunus* spp.). However, the species of this group included in our analyses, *Po. amygdalinum*, was not grouped among phyllachoraceous fungi but with *Trichosphaeriales* and *Xylariales* in the *Xylariomycetidae* (Fig. 2), as previously reported by Habibi et al. (2015). This exclusion from *Phyllachorales* is morphologically supported by the presence of sympodial proliferation of conidia rather than percurrent proliferation typical in species of *Phyllachorales*, and also by the accumulation of starch in the stromata of *Polystigma* spp., which is unusual in species of *Phyllachorales*. The other two species included in our analyses, *Po. pusillum* and *Polystigma* sp., were both placed in Clade I but not as closely related species. *Polystigma pusillum* has been related to the genus *Physalospora* (*Hyponectriaceae*) mainly due to the fact that microscopic features of the two genera are largely similar (Cannon 1991). As we mentioned before, our results confirm its placement within *Phyllachorales*, although its phylogenetic placement within the order is still uncertain. Another *Polystigma* sp. on *Poaceae* was grouped together with species of *P. graminis*. These results suggest that brightly coloured stromata evolved several times in the *Phyllachorales*. There are several *Phyllachora* spp. and *Stigmatula* spp. with poorly developed blackened tissue, so it is possible that species with brightly coloured stromata might be species of *Phyllachora* with reduced melanin pigmentation (Cannon 1996).

Clade II includes species growing on *Arecaceae*, which are characterised by the lack of a clypeus and a more developed pseudostroma, as well as, by saccate evanescent asci and ascospores with appendages or weak striations (Hyde et al. 1997). Species of two genera were included in the analyses, *Serenomyces* and *Coccolicola*, which formed two different subclades. *Serenomyces* spp. can be distinguished by the presence of individual ascomata with distinct necks, while *Coccolicola* spp. present multi-ostiolate ascomata without necks (Hyde & Cannon 1999).

Clade III includes species of *Phyllachorales* growing on plants of numerous families of eudicots and monocots, except the family *Poaceae*. All the species belonging to this clade have immersed perithecia and hyaline ascospores, mostly without

septa. Only one species, *Telimenia bicincta* on *Picramnia* spp., shows 3-septate ascospores but it is closely related to other species with aseptate ascospores. The number of septa in the ascospores has been used to separate *Telimenia* spp. from species of other genera, but our results show that the presence or absence of septa in the spores is not always systematically informative in the present context.

### Taxonomic implications

According to the current classification, Clades I and III include members of the accepted family *Phyllachoraceae* and Clade II corresponds to the family *Phaeochoaraceae*. The two traditionally accepted families in *Phyllachorales* can be distinguished morphologically by the lack of a clypeus and a more developed pseudostroma in species of *Phaeochoaraceae*, which is in contrast to mostly immersed perithecia beneath a clypeus typical for species of *Phyllachoraceae*; as well as by the presence of olivaceous to brownish ascospores with striate ornamentation in *Phaeochoaraceae* instead of smooth and hyaline ascospores typical of *Phyllachoraceae*.

Species of *Phyllachora* in the traditional sense are present in Clades I and III, so the genus needs to be redefined. Therefore, we revise the taxonomy of *Phyllachora* and the *Phyllachoraceae* to be consistent with the multi-gene phylogeny and the host relationships. The type species, *P. graminis*, forms part of a strongly supported clade of grass-associated species. This group is well studied (Orton 1944, Parbery 1967), morphologically homogenous, and should be considered *Phyllachora* s.str. The family *Phyllachoraceae* s.str. is emended and includes *Phyllachora* spp. that possess immersed perithecia and occur on *Poaceae*, and the erumpent perithecia genera *Camarotella* and *Coccodiella*. The suggested placement of *Po. pusillum* within *Phyllachoraceae* could not be confirmed.

Further *Phyllachora* species on other monocotyledonous and eudicotyledonous hosts form the strongly supported Clade III. The emendation of the family *Phyllachoraceae* and the genus *Phyllachora* require the recognition of a new family and at least one genus to accommodate the species clustering in Clade III. We propose to transfer these species to the genus *Telimenia* (Raciborski 1900), based on the phylogenetic position of *T. bicincta*, which is the oldest name available for species included in Clade III, and based on the type species of *Telimenia*, *T. erythrinae*, which does not occur on a species of *Poaceae* but on *Erythrina variegata* (*Fabaceae*). *Telimenia* was described for species with *Phyllachora* type stromata and 3-septate ascospores, instead of aseptate ascospores in typical *Phyllachora* spp. As mentioned above, molecular results indicate that the number of septa of the ascospore is not a reliable character to delimit genera in the present context; therefore we include species with septate as well as aseptate ascospores in the same genus. The examination of the type specimen of *T. erythrinae* and specimens of other *Telimenia* spp. showed that apart from the septation of the ascospores, stromatic characteristics of *Telimenia* spp. are similar to those of species of *Phyllachora* not growing on *Poaceae*, as has been pointed out by several authors (Raciborski 1900, Von Höhnelt 1911, Müller 1975, Barr 1977, Sivanesan 1987). The fact that *Telimenia* comprises species with ascomata similar to those of *Phyllachora* spp. means that the generic concept of *Telimenia* can be easily emended to include species considered *Phyllachora* spp. up to now. We assume that further molecular sequence data of the remaining *Phyllachora* spp. not occurring in *Poaceae* will not belong to *Phyllachora* s.str. but to Clade III due to their eudicotyledonous host. We propose the new family *Telimenaceae* for taxa belonging to Clade III.

Species that are proposed here as new combinations into the genus *Telimenia* correspond to species collected and sequenced by us. All of them have been compared with the corresponding type specimen and exhibited the same morphological characteristics. As no molecular sequence data could be obtained from the type material and most sequenced specimens were not collected at type localities, we mostly refrained from epitypification following recommendations by Hyde & Zhang (2008) as well as Zhang et al. (2013). Therefore, we decided to designate an epitype only for *T. bicincta*, which was obtained from the same location and host species as the type of this species.

Our findings strongly support the separation of Clades I and III from Clade II based on morphological, molecular, and host data, but it is difficult to identify morphological synapomorphies to separate Clade I from Clade III. Nevertheless, a careful re-examination of morphological characteristics of species classified in the families *Phyllachoraceae* s.str. (Clade I) and *Telimenaceae* (Clade III) revealed characteristics that allow distinguishing species of the two families morphologically: Stromata of tar spot fungi classified in the new family *Telimenaceae* are located either in subcuticular, epidermal, subepidermal, or immersed position, whereas stromata of species in *Phyllachoraceae* are either completely immersed in host tissue in the case of *Phyllachora* spp. on grasses or erumpent to superficial in species of the other genera. In *Phyllachora* spp. on grasses examined by Parbery & Langdon (1964) the pseudostroma was absent; they did not document any stromatic tissue apart from the clypeus. On the contrary, in *Telimenia* spp. on dicotyledonous plants, pseudostroma often is strongly developed, interfuses between adjacent stromata, and conspicuously expands into the host tissue.

### Evolution of parasite-host relationship

Members of the order *Phyllachorales* mainly infect monocotyledonous or dicotyledonous plant hosts. Our analyses show that the ancestor of *Phyllachorales* may have grown on monocotyledonous hosts. All three families recognized in this study include monocotyledonous plants as hosts. These results strongly suggest intimate relationships between *Phyllachorales* and monocotyledonous plants in the early evolution of the order. In general, long-term evolutionary dynamics or coevolution between hosts and their symbionts (parasitic or mutualistic relationships) operates in parallel by co-speciation or through speciation by host shifts. Co-speciation usually involves the speciation of a symbiont at the same time as another species, while host-shift speciation can occur when the symbiont moves to a new host on which the symbiont's immediate ancestor did not occur, and gives rise to new host-symbiont combinations (De Vienne et al. 2013).

Based on the host expansion strategy, two groups can be distinguished in the *Phyllachorales*, the family *Phaeochoaraceae* which apparently did not expand its host range outside *Areaceae*, and the families *Telimenaceae* and *Phyllachoraceae*, which expanded their host ranges to rather distant hosts. The facts that the phylogeny of *Phyllachorales* is not consistent with the phylogeny of their host plants, that a high number of distant host families are infected by phyllachoraceous fungi, and that several terminal groups of *Phyllachorales* species concentrate on hosts belonging to the same family, suggest that several host-shift speciation events followed by co-speciation might explain host-parasite patterns in *Phyllachoraceae* and *Telimenaceae*. We speculate that phyllachoraceous fungi first infected monocots, radiated on monocotyledonous hosts, and later expanded their host range to other dicotyledonous plant families by host jumps. For instance, in Clade I (*Phyllachoraceae*),



*Phyllachora* species are restricted to grasses but the family also includes the genera *Camarotella* and *Coccodiella* with species growing on other hosts. In the genus *Coccodiella*, *C. arundinariae* (not included in our analyses) is the only species which occurs on the monocotyledonous family *Poaceae*, specifically on bamboos in Far East Asia, but most of the species of the genus occur on the dicotyledonous family *Melastomataceae*. We hypothesize that the ancestor of *Coccodiella* on *Poaceae* probably infected a melastomataceous plant and expanded its host range within this family.

### Evolution of morphological characteristics

The position of the perithecia varies from completely immersed in the mesophyll of the leaf as in *Phyllachora* s.lat. spp. to completely superficial as in *Coccodiella* spp. (Fig. 1a–f). Several other genera have been erected based on this characteristic, i.e., *Camarotella* spp. with erumpent perithecia, *Trabutia* spp. with subcuticular perithecia or *Catacauma* spp. with perithecia inserted between the clypeus and the epidermis. However, the location of the perithecia in the leaf has been suggested to be greatly influenced by the consistency of the host tissue (Parbery & Langdon 1964, Cannon 1991), and therefore not very reliable as a taxonomical criterion. The reconstruction presented here suggests that the ancestral state for *Phyllachorales* was completely immersed perithecia, which apparently was lost in the family *Phaeocharaceae* and evolved to erumpent or superficial perithecia in some members of *Phyllachoraceae*. In the genus *Telimenia* the position of perithecia varies from immersed to subepidermal. Based on our results, when the perithecia are superficial or erumpent, as in the genera *Coccodiella* and *Camarotella*, this characteristic is reliable to define genera, while subepidermal and subcuticular positions of perithecia might be dependent on the texture and anatomy of the host tissue.

Our data also indicate that phyllachoraceous fungi growing on palms form a distinctive group, probably due to the very particular anatomical characteristics of palms. In these fungi, the expansion of the stomata and the shape of the perithecia are affected by leaves with closely spaced, strongly lignified, parallel vascular bundles, typical of *Arecaceae*. Our phylogeny shows that palm-inhabiting species have evolved independently at least three times within *Phyllachorales*, as *Arecaceae* is the only host family occurring in all three fungal families. According to Hyde & Cannon (1999), there are three different types of stomata in phyllachoraceous fungi on palms, one elongated and erumpent as in *Camarotella* spp. (Clade I, *Phyllachoraceae*), another one inserted between the outermost layers of the host tissue, as in family *Phaeocharaceae* (Clade II), and a few species having immersed perithecia, as in *Telimenaceae* (Clade III), which are confined to species of palms with less lignified leaf tissue. Our results do not confirm that the ancestor of *Phyllachorales* was growing on a palm. Therefore, more research is needed to elucidate the role of palm-inhabiting species in the evolutionary history of *Phyllachorales*.

Most species within *Phyllachorales* produce black stomata caused by dense fungal cells with melanin deposits, the only exception being *Polystigma* spp. Our analyses suggest that the presence of black stomata is the ancestral state in *Phyllachorales*, and that brightly coloured stomata evolved at least twice in the order.

The clypeus is a shield of black fungal cells located above the perithecia, which is restricted to the epidermal cells of the host and is thought primarily to protect the developing tissues from damage caused by UV radiation or to absorb heat from the sun promoting growth (Durrell & Shields 1960, Sherwood 1981). Species of *Phyllachorales* share the presence of a clypeus as a synapomorphy, which apparently was lost only once in the

*Phaeocharaceae*, thus, the clypeus is thought to represent an evolutionarily stable characteristic in the order.

### Conclusions and future work

This study demonstrates the monophyly of *Phyllachorales* and its placement in *Sordariomycetidae* with *Boliniales* as a sister group. Although several genera, which possibly do not belong to *Phyllachorales*, were not represented in our dataset, it is clear that there is a core group representing this order. The phylogenetic relationships within the order are partially elucidated. The placement of *Phaeocharaceae* within *Phyllachorales* is confirmed, however, more sampling in the three families is necessary to better assess their internal relationships. Our data also supports the split of the family *Phyllachoraceae* and the genus *Phyllachora*, and the establishment of the additional family *Telimenaceae* and the genus *Telimenia*. With monophyly demonstrated, efforts should be made to find characteristics that help to distinguish between families. In this study, apart from the molecular and ecological distinctions, few morphological synapomorphies were detected to differentiate the families *Telimenaceae* and *Phyllachoraceae*. Potentially informative characteristics that should be evaluated are the ascus wall and the morphology of the ascus apex. Additional morphological and ultrastructural work by electron microscopy will contribute to our understanding of the asci. Other valuable characteristics might be the asexual/spermatogonial states of *Phyllachorales* and the morphology of the haustoria. Also, additional molecular markers are necessary for a profound phylogenetic study of some specific clades. Although our study contains a comprehensive dataset, it is still not possible to clearly circumscribe the family *Phyllachoraceae* and to elucidate the monophyly of the genera within *Phyllachorales*. DNA should be generated for species of *Phyllachora* on grasses and for several other genera that have been erected historically based on single characteristics of ascospores, i.e., *Apiosphaeria*, *Ophiodothella*, *Sphaerodothis*, *Stigmochora*, *Telimenochora*. Obtaining fresh specimens of these fungi to place them within a molecular framework should be an important objective of future studies, to prove the validity of the characteristics used to delimit them. Further molecular analyses including sequences of more species, from new locations and host plants, will contribute to an understanding of the evolution of host ranges. We predict that this re-evaluation will produce the reassessment of several genera.

The results of ancestral state reconstruction analyses rely on the phylogeny of the present analysis that includes only a small fraction of species known for the corresponding systematic relationships. Therefore, the ancestral state reconstructions are not certain and the true number of state changes is probably underestimated. A broader sampling will probably reveal further state changes specially regarding subsequent host jumps and also concerning the position of the perithecia in the leaves in species belonging to *Phyllachoraceae* and *Telimenaceae*.

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**Appendix 1** Character matrix used for ancestral state reconstruction analyses of members of *Phyllachorales*.

Species	Voucher	Host <sup>a</sup>	Perithecial position <sup>b</sup>	Clypeus <sup>c</sup>	Stromata colour <sup>d</sup>	Morphological observations based on
<i>Camarotella costaricensis</i>	MM-149	0	1	0	0	specimen
	MM-21	0	1	0	0	specimen
<i>Camarotella</i> sp.	MM-27	0	1	0	0	specimen
<i>Coccodiella melastomatum</i>	CMU78543	1	2	0	0	Léveillé 1845 (as <i>Sphaeria melastomatum</i> )
<i>Coccodiella miconiae</i>	ppMP1342	1	2	0	0	specimen
<i>Coccodiella miconiicola</i>	TH571	1	2	0	0	specimen
<i>Coccodiella</i> sp.	MM-165	1	2	0	0	specimen
<i>Coccodiella toledoï</i>	Unknown	1	2	0	0	Chardón & Toro 1934 (as <i>Bagnisiopsis toledoï</i> )
<i>Coccolicola californica</i>	F59034	0	3	1	0	specimen
	F59038	0	3	1	0	specimen
<i>Phyllachora graminis</i>	AF257111	0	0	0	0	Parbery 1967
	DAOM240981	0	0	0	0	Parbery 1967
	RoKi3084	0	0	0	0	specimen
	MM-166	0	0	0	0	specimen
	UME31349	0	0	0	0	Parbery 1967
<i>Phyllachora maydis</i>	BPI893231	0	0	0	0	Parbery 1967
<i>Phyllachora</i> sp. 1	MM-130	0	0	0	0	specimen
<i>Phyllachora</i> sp. 2	MM-128	0	0	0	0	specimen
<i>Phyllachora</i> sp. 2	MM-129	0	0	0	0	specimen
<i>Phyllachora</i> sp. 3	MM-135	0	0	0	0	specimen
<i>Phyllachora</i> sp. 3	MM-78	0	0	0	0	specimen
<i>Phyllachora</i> sp. 3	MM-98	0	0	0	0	specimen
<i>Phyllachora</i> sp. 3	SO-07	0	0	0	0	specimen
<i>Phyllachora</i> sp. 4	RMB1061	0	0	0	0	specimen
<i>Polystigma pusillum</i>	MM-113	1	0	0	1	specimen
	MM-147	1	0	0	1	specimen
	MM-19	1	0	0	1	specimen
<i>Polystigma</i> sp.	MM-163	0	0	0	1	specimen
<i>Serenomyces phoenicis</i>	PLM314	0	3	1	0	specimen
	PLM315	0	3	1	0	specimen
<i>Telimena aequatoriensis</i>	SO-05	1	0	0	0	specimen
<i>Telimena bicincta</i>	MM-133	1	0	0	0	specimen
	MM-108	1	0	0	0	specimen
<i>Telimena canafistulae</i>	MM-13	1	0	0	0	specimen
<i>Telimena engleri</i>	MM-153	0	0	0	0	specimen
	MM-159	0	0	0	0	specimen
	TH551	0	0	0	0	specimen
	SO-09	0	0	0	0	specimen
<i>Telimena leeeae</i>	TH549	1	0	0	0	specimen
<i>Telimena picramniae</i>	MM-05	1	0	0	0	specimen
<i>Telimena</i> sp. 1	MM-57	1	0	0	0	specimen
<i>Telimena</i> sp. 2	MM-143	1	0	0	0	specimen
<i>Telimena</i> sp. 2	MM-144	1	0	0	0	specimen
<i>Telimena</i> sp. 3	MM-92	1	0	0	0	specimen
<i>Telimena</i> sp. 4	MM-88	1	0	0	0	specimen
<i>Telimena</i> sp. 5	MM-47	1	0	0	0	specimen
<i>Telimena</i> sp. 6	SO-14	0	0	0	0	specimen
<i>Telimena</i> sp. 6	SO-21	0	0	0	0	specimen
<i>Telimena</i> sp. 6	SO-22	0	0	0	0	specimen
<i>Telimena ulei</i>	SO-12	0	0	0	0	specimen
	TH574	0	0	0	0	specimen
<i>Telimena zanthoxylicola</i>	TH550	1	0	0	0	specimen

<sup>a</sup> 0 = monocotyledonous host plant; 1 = dicotyledonous host plant.  
<sup>b</sup> 0 = immersed in the mesophyl of the leaf; 1 = erumpent; 2 = superficial; 3 = subcuticular.  
<sup>c</sup> 0 = present; 1 = absent.  
<sup>d</sup> 0 = black; 1 = bright coloured.