



Article

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## *Neophyllachora* gen nov. (Phyllachorales), three new species of *Phyllachora* from Poaceae and resurrection of *Polystigmataceae* (Xylariales)

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### Abstract

We collected six “tar spot” disease specimens from various hosts and these were subjected to morpho-phylogenetic studies. In this paper, a new genus, *Neophyllachora* is introduced to accommodate *N. cerradensis*, *N. myrciae*, *N. myrciariae*, *N. subcircinans* and *N. truncatispora*, which are related to *Phyllachora* species but constitutes an independent strongly supported monophyletic clade within *Phyllachoraceae* of the Phyllachorales. Three novel *Phyllachora* species; *P. chloridis*, *P. cynodonticola* and *P. panicola* on Poaceae are also introduced. Phenotypic comparisons and phylogenetic analysis of partial SSU, LSU and ITS sequence data with homologous taxa, confirm the placement of the novel species in *Phyllachoraceae*. The family *Polystigmataceae* is re-established to accommodate *Polystigma* within the order Xylariales. The asexual morph of *Polystigma rubrum* was re-collected from Russia and is provided as a reference specimen with a description, illustrations and molecular data. Further studies with multiple gene analysis are recommended to provide a natural and stable classification system for members of Phyllachorales.

**Key words** – biotrophs – phylogeny – tar spots – taxonomy

## Introduction

Members of the family *Phyllachoraceae* are obligate biotrophs known to be minor pathogens, and facilitate a pathway for secondary infection by other more severe pathogenic organisms (Pearce et al. 1999). *Phyllachoraceae* was introduced by Theissen & Sydow (1915) and referred to different orders, including Dothideales (Horst 1990), Sphaeriales (Nannfeldt 1932, Miller 1949, Muller & Arx 1962, Wehmeyer 1975), Xylariales (Luttrell 1951, Barr 1990), Glomerellales (Chadefaud 1960, Locquin 1984), Phyllachorales (Barr 1976a, b, 1983), Polystigmatales (Eriksson 1982, Hawksworth et al. 1983) and Diaporthales (Cannon 1988). Maharachchikumbura et al. (2016) placed the order Phyllachorales with two families, *Phaeochoraceae* and *Phyllachoraceae*, in the subclass Sordariomycetidae and this was confirmed by later studies (Maharachchikumbura et al. 2016, Hongsanan et al. 2017). A recent study by Mardones et al. (2017) proposed a new family *Telimenaceae* with *Telimenia erythrinae* as the type species, resulting in three families in the Phyllachorales. The number of recognized genera within this diverse family varies according to the authority. Hawksworth (1985) recognized 23 genera, whereas Barr (1990) provided a key to genera in *Phyllachoraceae*, including only 12 genera. Eriksson & Hawksworth (1993) recognized 39 genera; Hawksworth et al. (1995) accepted 42 genera, while Pearce & Hyde (2001) introduced a novel genus *Parberya*. *Phyllachoraceae* currently comprises 59 genera (Maharachchikumbura et al. 2016, Mardones et al. 2017) which are believed to have evolved at approximately 217 MYA (Hongsanan et al. 2017). Species of *Phyllachoraceae* have been documented from a variety of plant host families (Pearce & Hyde 1993, Cannon 1997, Pearce et al. 2000, 2001) and most taxa have been presumed to be host-specific (Pearce et al. 1999). Members of *Phyllachoraceae* can be recognized by ascohymenial development with paraphyses, thin-walled asci, which might have an apical ring and ascospores that are often hyaline and 1-celled (Cannon 1991, Maharachchikumbura et al. 2016). The asexual morphs of *Phyllachoraceae* are coelomycetes, spermatial or disseminative (Hawksworth et al. 1995).

The genus *Phyllachora* was introduced based on a herbarium label in Fuckels exsiccate series 'Fungi Rhenani' with a single species, *P. agrostis* (Fuckel 1867 in Cannon 1991), synonymized as *Scirrhia agrostis* in Dothideales (Eriksson 1967). Later, *Phyllachora* was lectotypified with *P. graminis* [Pers.] Fuckel) as generic type (Clements & Shear 1931). Currently *Phyllachora* is recognized as the largest genus in this family with about 1500 species epithets (Maharachchikumbura et al. 2016, Index Fungorum 2017). It comprises species that grow immersed in a clypeate pseudostroma in leaf tissues, varying from a subcuticular or intra-epidermal, to a generalized infection of the entire section of the mesophyll, inducing characteristic black shiny superficial symptoms, commonly known as tar spots (Santos et al. 2016). A large number of *Phyllachora* species have been given names based on host association and this may not reflect the actual number of species (Cannon 1988). There have been several morphological studies of *Phyllachora* on various groups of host plants. For example, leaf-spots on grasses and sedges in northern regions (Seaver 1928), *Phyllachora* species causing tar spot on *Leguminosae* (Cannon 1991), *Duranta* spp. in the tropics (Hanlin & Tortolero 1991), small scabby leaf spots or "lixa-pequena" on coconut palms in Brazil (Subileau et al. 1993), leaf-spots on the plant family Asclepiadaceae (Pearce et al. 1999), phyllachoraceous species from *Arecaceae* (Hyde & Cannon 1999), *Phyllachora shiraiana* complex from *Bambusa arnhemica* (Pearce et al. 2000), *Phyllachoraceae* species on hosts in the family Proteaceae and tar spots on grasses in Australia (Sivanesan & Shivas 2002) and *Phyllachora thysanolaenae* on *Thysanolaena maxima* and *Phyllachora vetiveriana* on *Chrysopogon zizanioides* in Thailand (Tamakaew et al. 2017). However, most of these studies are based on morphological data. There is still some taxonomic confusion with the taxonomy of *Phyllachora* given that species have been reported to be polyphyletic (Santos et al. (2016).

*Polystigma* is a biotrophic genus also included at times in *Phyllachoraceae*, which causes reddish-brown or purple leaf spots on living leaves mostly on *Prunus* species. Accurate morphological identification of the species that cause these diseases remains uncertain, but is thought to be a close

relative of *Phyllachora*, as only a few distinguishing characters remain between the two genera apart from stromatal pigmentation (Cannon 1996). *Polystigma* asexual morphs have conidiogenous cells, which proliferate sympodially, rather than percurrently, which is a common characteristic of asexual morphs of *Phyllachoraceae* (Cannon 1996, Habibi et al. 2015). The type, *Polystigma rubrum* is characterized by typically swollen, red/orange/brown (not purple) stromata, bordered by apparently healthy leaf tissues that produce distinctive hooked or curved conidia (<http://fungi.myspecies.info/content/lost-found-fungi-project>, 2015). Historically this species was documented from 30 counties in Great Britain and Ireland, found abundantly along the east and west coasts of England and Wales (<http://fungi.myspecies.info/content/lost-found-fungi-project>, 2015). Farr & Rossman (2016) recorded this species as widely distributed throughout the world. In a study by Habibi et al. (2015) *Po. amygdalinum* and *Po. rubrum* grouped in Xylariomycetidae and in Mardones et al. (2017) *Po. pusillum* and *Polystigma* sp. clustered with *Phyllachora* species, while other *Polystigma* species did not group among other phyllachoraceous taxa, but with Trichosphaeriales and Xylariales in the Xylariomycetidae.

The aim of this study is a morpho-molecular evaluation of 30 spots of six “tar spot” disease specimens collected in China and Thailand. We introduce a novel genus, *Neophyllachora*, in *Phyllachoraceae*, to accommodate some *Phyllachora* taxa which are phylogenetically distinct from the type species. Three novel species of *Phyllachora* are introduced along with a re-assessment of *Phyllachoraceae* species. *Polystigmataceae* is re-established with descriptions, illustrations and molecular data from *Po. rubrum* which groups within the order Xylariales.

## Materials and Methods

### *Sample collections and examination*

Fresh materials were collected from China, Russia and Thailand during January 2015 to December 2016. Samples were examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters of taxa were observed and images were captured under a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Observations and photographs were made from squash mounts of fresh fruiting bodies mounted in water and stained with Melzer’s reagent. Measurements were taken with the Tarosoft (R) Image Frame Work. Adobe Photoshop CS5 version 10.0 software was used to prepare the Fig.s. Herbarium specimens were deposited in the Mae Fah Luang University Herbarium (MFLU) and Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS). Faces of fungi and Index Fungorum numbers were registered according to Jayasiri et al. (2015), Index Fungorum (2017). New species are established based on recommendations outlined by Jeewon & Hyde (2016).

### *DNA extraction, PCR amplification and sequencing*

Genomic DNA was extracted from fruiting bodies using an E.Z.N.A. ® Forensic DNA kit (D3591- 01, Omega Bio-Tek) following the manufacturer’s protocol. DNA amplification was performed by polymerase chain reaction (PCR). The partial large subunit nuclear rDNA (28S, LSU) was amplified with primer pair LROR and LR5 (Vilgalys & Hester 1990). Primer pair NS1 and NS4 was used to amplify the partial small subunit nuclear rDNA (18S, SSU) (White et al. 1990) and the primer pair ITS5 and ITS4 was used to amplify the internal transcribed spacer regions (ITS) (White et al. 1990). Amplifications was performed in 25 µl of PCR mixtures containing 9.5 µl ddH<sub>2</sub>O, 12.5 µl 2 × PCR Master Mix, 1 µl of DNA template and 1 µl of each primer (10 µM). The PCR thermal cycle program was an initial step of 3 mins at 94°C, followed by 35 cycles of 30 sec at 94°C, 58 sec at 30°C, and 1 min at 72°C, with a final extension of 10 mins at 72°C. The PCR products were observed on 1% agarose electrophoresis gels stained with 4S green stain.

## Phylogenetic analysis

**Table 1** GenBank and culture collection accession numbers of isolates included in this study. Sequences generated in this study are in blue.

Taxon	Strain	GenBank Accessions		
		LSU	SSU	ITS
<i>Achaetomium strumarium</i>	IMI 082624	AJ312098	-	-
<i>Albertiniella polyporicola</i>	NBRC 30914	AB178271	-	AB278196
<i>Ambrosiella ferruginea</i>	CBS 460 82	EU825651	KR673890	KC305146
<i>Amphisphaeria umbrina</i>	HKUCC 994	AF452029	AY083811	-
<i>Amplistroma caroliniana</i>	BEO9923	FJ532377	-	-
<i>Amplistroma erinaceum</i>	AH 43902	KC907374	-	NR145379
<i>Amplistroma longicollis</i>	AH37870	HQ901790	-	-
<i>Annulatascus velatisporus</i>	HKUCC 3701	AF132320	-	-
<i>Annulusmagnus triseptatus</i>	CBS 128831	GQ996540	JQ429242	-
<i>Apiorhynchostoma curreyi</i>	UAMH 11088	NG042715	-	NR120207
<i>Apiospora montagnei</i>	AFTOL-ID 951	DQ471018	FJ190614	-
<i>Arachnocrea stipata</i>	TFC 97-43	AF160227	-	-
<i>Arthrimum phaeospermum</i>	HKUCC 3395	AY083832	AY083816	-
<i>Ascitendus austriacus</i>	MR 2936	GQ996539	GQ996542	-
<i>Ascothailandia grenadoidia</i>	NB 2010a	GQ390267	GQ390252	GQ390282
<i>Ascovaginospora stellipala</i>	P5-13A	ASU85088	-	-
<i>Australiasca queenslandica</i>	BRIP 24607	HM237324	-	HM237327
<i>Bartalinia robillardoides</i>	CBS 122705	KJ710438	-	NR126145
<i>Beltrania pseudorhombica</i>	CPC 23656	KJ869215	-	KJ869158
<i>Bionectria ochroleuca</i>	AFTOL-ID 187	DQ862027	DQ862044	-
<i>Bisconiauxi anummularia</i>	MUCL51395	KT281894	-	KY610382
<i>Bombardia bombardia</i>	AFTOL-ID 967	DQ470970	-	DQ471021
<i>Cainia graminis</i>	CBS 136.62	AF431949	AF431952	-
<i>Calosphaeria pulchella</i>	JF 03200	AY761075	AY761071	-
<i>Camarotella costaricensis</i>	MM-149	KX430484	KX451863	KX451913
<i>Camarotella costaricensis</i>	MM-21	KX430490	KX451851	KX451900
<i>Camarotella</i> sp.	MM-27	KX430492	KX451852	KX451901
<i>Canalisporium caribense</i>	SS038397	GQ390269	GQ390253	GQ390284
<i>Carpoligna pleurothecii</i>	CBS 101580	GU017318	JQ429247	JQ429147
<i>Catabotrys deciduum</i>	SMH3436	AY346268	-	-
<i>Cephalotheca foveolata</i>	UAMH11631	KC408398	-	KC408422
<i>Chaetomidium galaicum</i>	CBS 113678	FJ666361	-	-
<i>Chaetosphaerella fusca</i>	GKML124N	FJ968967	-	-
<i>Claviceps purpurea</i>	AEG 97 2	AF543789	AF543765	-
<i>Clypeosphaeria uniseptata</i>	HKUCC 6349	AY083830	DQ810255	-
<i>Coccodiella melastomatum</i>	54964	-	CMU78543	-
<i>Coccodiella melastomatum</i>	CMU78543	-	U78543	-
	PMAPAN M			-
<i>Coccodiella miconiae</i>	0141106	-	GU233440	-
<i>Coccodiella miconiae</i>	MP1342	KX430506	KX451871	-

**Table 1** Continued.

Taxon	Strain	GenBank Accessions		
		LSU	SSU	ITS
<i>Coccodiella miconiicola</i>	TH571	KX430512	KX451880	-
<i>Coccodiella</i> sp.	MM-165	KX430488	KX451865	KX451917
<i>Coccodiella toledoii</i>	Unknown	-	U78544	-
<i>Cocoicola californica</i>	F59034	KX430468	KX451866	KX451918
<i>Cocoicola californica</i>	F59038	KX430469	KX451867	KX451919
<i>Colletotrichum gloeosporioides</i>	LC0555	JN940412	JN940356	JN943090
<i>Coniooessia maxima</i>	Co117	GU553344	-	NR137751
<i>Coniooessia nodulisporioides</i>	CBS 281.77	AJ875224	AJ875185	-
<i>Coniochaeta ligniaria</i>	C8	AY198388	AY198389	AY198390
<i>Conioscypha lignicola</i>	CBS 335.93	AY484513	JQ437440	-
<i>Copromyces</i> sp.	CBS 386.78	AY346277	DQ471007	-
<i>Cordana abramovii</i>	PE 0053-24a	KF833358	-	-
<i>Cordana inaequalis</i>	CBS 508.83	HE672157	-	NR145363
<i>Cordana mercadiana</i>	FMR:11828	HE672165	-	HE672154
<i>Cordana pauciseptata</i>	CBS 121804	HE672160	-	HE672149
<i>Cordyceps cardinalis</i>	OSC 93609	AY184962	AY184973	-
<i>Cryptadelphia groenendalensis</i>	SMH3767	EU528001	-	-
<i>Cryptendoxyla hypophloia</i>	WM10 89	HQ014708	-	-
<i>Diatrype disciformis</i>	AFTOLID 927	DQ470964	DQ471012	-
<i>Diatrype whitmanensis</i>	ATCC MYA-4417	FJ430587	-	-
<i>Discostroma</i> sp.	HKUCC 1004	AF382380	AY083814	-
<i>Dothidea sambuci</i>	DAOM 231303	NG027611	NR111220	AY883094
<i>Duradens</i> sp.	SMH1708	AY780068	AY761067	-
<i>Endoxyla operculata</i>	UAMH 11085	NG042718	-	NR120209
<i>Etheiophora blepharospora</i>	JK5397A	EF027723	-	EF027717
<i>Exserticlava vasiformis</i>	TAMA 450	AB753846	-	-
<i>Falcocladium sphaeropedunculatum</i>	CBS 111292	JF831933	JF831929	JF831938
<i>Falcocladium thailandicum</i>	CBS 121717	JF831934	JF831930	JF831939
<i>Flammocladiella aceris</i>	CPC 24422	KR611901	-	KR611883
<i>Fragosphaeria purpurea</i>	CBS 133.34	AB189154	AF096176	AB278192
<i>Gelasinospora tetrasperma</i>	CBS 178.33	-	-	NR077163
<i>Gnomonia gnomon</i>	CBS199.53	AF408361	-	-
<i>Gondwanamyces capensis</i>	CMW 997	KM495391	-	EU660439
<i>Graphostroma platystoma</i>	AFTOL-ID 1249	DQ836906	DQ836900	-
<i>Halosphaeria appendiculata</i>	CBS 197.60	HAU46885	HAU46872	-
<i>Hyponectria buxi</i>	UME 31430	AY083834	AF130976	-
<i>Iodosphaeria tongrenensis</i>	MFLU 15-0393	KR095283	KR095284	KR095282
<i>Jattaea prunicola</i>	STE-U 6201	EU367456	EU367462	NR135946
<i>Juncigena adarca</i>	JK5235A	EF027726	EF027719	-
<i>Koralionastes ellipticus</i>	JK5771	EU863583	EU863580	-
<i>Koralionastes ellipticus</i>	JK5769	EU863585	EU863581	-
<i>Lasiosphaeria ovina</i>	SMH4605	AY436413	-	AY587923.
<i>Lecythophora luteoviridis</i>	CBS 206 38	FR691987	-	DQ404354

**Table 1** Continued.

Taxon	Strain	GenBank Accessions		
		LSU	SSU	ITS
<i>Lecythothecium duriligini</i>	CBS 101317	AF261071	-	-
<i>Leucostoma niveum</i>	AR3413	AF362558	-	-
<i>Lindra thalassiae</i>	AFTOL-ID 413	DQ470947	DQ470994	DQ491508
<i>Lopadostoma turgidum</i>	LT2	-	-	KC774618
<i>Lulworthia fucicola</i>	ATCC 64288	AY878965	AY879007	-
<i>Magnaporthe salvinii</i>	M21	JF414887	JF414862	JF414838
<i>Melanochaeta hemipsila</i>	SMH2125	AY346292	-	-
<i>Melogramma campylosporium</i>	JF440978	-	-	JF440978
<i>Microascus trigonosporus</i>	AFTOL-ID 914	DQ470958	DQ471006	DQ491513
<i>Microdochium phragmitis</i>	CBS 423.78	KP858948	-	KP859012
<i>Nectria cinnabarina</i>	CBS 114055	KU382228	-	-
<i>Neophyllachora cerradensis</i>	UB15626	-	-	KC683454
<i>Neophyllachora cerradensis</i>	UB16014	-	-	KC683455
<i>Neophyllachora cerradensis</i>	UB21823	-	KC740651	KC683470
<i>Neophyllachora cerradensis</i>	UB21908	-	KC740623	KC683471
<i>Neophyllachora myrciae</i>	UB21292	-	KC902620	KC683463
<i>Neophyllachora myrciae</i>	UB22192	-	KC740631	KC683476
<i>Neophyllachora myrciariae</i>	UB21781	-	-	KC683469
<i>Neophyllachora subcircinans</i>	UB09748	-	-	KC683441
<i>Neophyllachora subcircinans</i>	UB21238	-	-	KC683461
<i>Neophyllachora subcircinans</i>	UB21347	-	-	KC683466
<i>Neophyllachora subcircinans</i>	UB21747	-	KC902622	KC683467
<i>Neophyllachora truncatispora</i>	UB14083	-	KC740652	KC683448
<i>Neurospora crassa</i>	MUCL 19026	AF286411	-	-
<i>Niesslia exilis</i>	CBS 357.70	AY489718	AY489686	-
<i>Nitschkia tetraspora</i>	GKML148N	FJ968987	-	-
<i>Ophioceras dolichostomum</i>	HKUCC 10113	DQ341507	DQ341485	-
<i>Ophioceras dolichostomum</i>	CBS 114926	JX134689	JX134663	NR120171
<i>Ophiocordyceps sinensis</i>	YN09 64	JX968033	JX968028	JQ325141
<i>Ophiodiaporthe cyatheae</i>	YMJ 1364	JX570891	JX570890	JX570889
<i>Ophiodothella vaccinii</i>	ATCC 36333	-	U78777	-
<i>Ophiostoma stenoceras</i>	AFTOL-ID 1038	DQ836904	DQ836897	-
<i>Parapleurotheciopsis inaequiseptata</i>	MUCL 4108	EU040235	-	EU040235
<i>Pestalotiopsis theae</i>	SAJ-0021	JN940838	JN940785	JN943623
<i>Phaeoacremonium novae-zealandiae</i>	WIN 113BI	AY761081	AY761069	NR136064
<i>Phlogicylindrium eucalyptorum</i>	CBS 111689	KF251708	-	KF251205
<i>Phlogicylindrium uniforme</i>	CBS 131312	JQ044445	-	JQ044426
<i>Phyllachora chloridis</i>	<b>MFLU 15-0173</b>	<b>MF197499</b>	<b>MF197505</b>	<b>KY594026</b>
<i>Phyllachora chloridis</i>	<b>MFLU 16-2980</b>	<b>MF197500</b>	<b>MF197506</b>	<b>KY594027</b>
<i>Phyllachora chrysopagonii</i>	MFUH 16 -2096	MF372146	-	MF372145
<i>Phyllachora cynodonticola</i>	<b>MFLU 16-2978</b>	<b>MF197502</b>	<b>MF197508</b>	<b>KY594025</b>
<i>Phyllachora cynodonticola</i>	<b>MFLU 16-2977</b>	<b>MF197501</b>	<b>MF197507</b>	<b>KY594024</b>

**Table 1** Continued.

Taxon	Strain	GenBank Accessions		
		LSU	SSU	ITS
<i>Phyllachora graminis</i>	101486	-	-	AF257111
<i>Phyllachora graminis</i>	DAOM 240981	-	-	HQ317550
<i>Phyllachora graminis</i>	UME 31349	-	AF064051	-
<i>Phyllachora graminis</i>	RoKi3084	-	KX451872	-
<i>Phyllachora graminis</i>	MM-166	-	KX451869	KX451920
<i>Phyllachora graminis</i>	TH544	KX430508	KX451873	-
<i>Phyllachora maydis</i>	BPI 893231	-	-	KU184459
<i>Phyllachora panicicola</i>	<b>MFLU16-2979</b>	<b>MF197503</b>	<b>MF197504</b>	<b>KY594028</b>
<i>Phyllachora qualeae</i>	UB21145	-	-	KT380955
<i>Phyllachora qualeae</i>	UB 21159	-	-	KU682781
<i>Phyllachora qualeae</i>	UB 21771	-	-	KU682780
<i>Phyllachora</i> sp.	MM-130	-	KX451883	-
<i>Phyllachora</i> sp.	MM-128	-	KX451859	KX451908
<i>Phyllachora</i> sp.	MM-129	-	KX451860	KX451909
<i>Phyllachora</i> sp.	MM-135	-	KX451885	-
<i>Phyllachora</i> sp.	MM-78	-	KX451853	-
<i>Phyllachora</i> sp.	MM-98	KX430502	KX451856	-
<i>Phyllachora</i> sp.	MM-134	KX430479	KX451884	-
<i>Phyllachora</i> sp.	SO-07	-	KX451890	-
<i>Phyllachora</i> sp.	RMB1061	-	KX451870	KX451921
<i>Plagiostoma euphorbiae</i>	CBS 340.78	AF408382	-	DQ323532
<i>Pleurostoma ootheca</i>	CMU 23858	AY761079	AY761074	-
<i>Pleurostomophora repens</i>	CBS H7594	AY761078	AY761067	-
<i>Pleurothecium semifecundum</i>	CBS 131271	-	JQ429254	NR111710
<i>Polystigma amygdalinum</i>	EA-1	-	-	KC756360
<i>Polystigma amygdalinum</i>	1276059	KM111540	KM111539	-
<i>Polystigma pusillum</i>	MM-113	KX430474	KX451858	KX451907
<i>Polystigma pusillum</i>	MM-147	-	KX451862	-
<i>Polystigma pusillum</i>	MM-19	KX430489	KX451850	KX451899
<i>Polystigma rubrum</i>	<b>MFLU15-3091</b>	<b>MF981079</b>	-	<b>KY594023</b>
<i>Polystigma</i> sp.	Rub1	-	-	KC966927
<i>Polystigma</i> sp.	MM163	KX430487	KX451864	KX451916
<i>Pseudomassaria chondrospora</i>	PC1	-	-	JF440982
<i>Pseudomassaria chondrospora</i>	MFLUCC 15-0545	KR092779	-	KR092790
<i>Pseudoneurospora amorphoporcata</i>	CBS 626.80	FR774287	-	-
<i>Pyricularia borealis</i>	CBS 461.65	KM009150	KM009210	NR145384
<i>Reticulascus clavatus</i>	CBS 125296	GU180643	GU180622	NR137741
<i>Robillarda sessilis</i>	CBS 114312	KR873284	-	NR132928
<i>Scortechiniellopsis leonensis</i>	GKM1269	FJ968993	-	-
<i>Seimatosporium cornii</i>	MFLUCC 14-0467	KR559739	KR559741	KT162918
<i>Selenodriella fertilis</i>	CBS 772.83	KP858992	-	KP859055
<i>Serenomyces phoenicis</i>	PLM315	KX430505	KX451886	-
<i>Seynesia erumpens</i> new	SMH 1291	AF279410	AF279409	-

**Table 1** Continued.

Taxon	Strain	GenBank Accessions		
		LSU	SSU	ITS
<i>Sordaria fimicola</i>	CBS 508.50	-	-	AY681188
<i>Stachybotrys chlorohalonata</i>	UAMH6417	AY489712	AY489680	AF206273
<i>Telimena aequatoriensis</i>	SO-05	KX430505	KX451886	-
<i>Telimena bicincta</i>	MM-133	-	KX451889	-
<i>Telimena bicincta</i>	MM-108	KX430478	KX451861	KX451910
<i>Telimena canafistulae</i>	MM-13	-	KX451857	KX451906
<i>Telimena engleri</i>	MM-153	KX430477	KX451849	KX451898
<i>Telimena engleri</i>	MM-159	-	KX451888	KX451914
<i>Telimena engleri</i>	TH551	-	-	KX451915
<i>Telimena engleri</i>	SO-09	KX430511	KX451875	KX451895
<i>Telimena leae</i>	TH549	-	-	KX451934
<i>Telimena picramniae</i>	MM-05	KX430509	KX451874	-
<i>Telimena</i> sp.	MM-143	KX430470	KX451848	KX451896
<i>Telimena</i> sp.	MM-144	-	KX451887	KX451911
<i>Telimena</i> sp.	MM-92	-	-	KX451912
<i>Telimena</i> sp.	MM-88	KX430501	KX451855	KX451905
<i>Telimena</i> sp.	MM-47	KX430499	KX451854	KX451904
<i>Telimena</i> sp.	SO-14	-	-	KX451902
<i>Telimena</i> sp.	SO-21	-	KX451892	KX451936
<i>Telimena</i> sp.	SO-22	-	KX451893	KX451937
<i>Telimena ulei</i>	SO-12	-	KX451891	KX451935
<i>Telimena ulei</i>	TH574	-	KX451877	-
<i>Telimena zanthoxylicola</i>	TH550	KX430510	KX451879	-
<i>Tilachlidium brachiatum</i>	CBS 363.97	KM231719	-	KM231838
<i>Togninia griseoolivacea</i>	STE U 5966	-	EU128058	NR135939
<i>Torpedospora radiata</i>	AFTOL-ID 751	DQ470951	DQ470999	-
<i>Umbrinosphaeria caesariata</i>	CBS 102664	AF261069	-	-
<i>Vialaea mangiferae</i>	MFLUCC 12-0808	KF724975	-	KF724974
<i>Vialaea minutella</i>	BRIP 56959	KC181924	-	KC181926
<i>Xylaria hypoxylon</i>	STMA 07069	KM186301	-	-
<i>Xylaria polymorpha</i>	MUCL 49884	KY610464	-	KY610408

Purification and sequencing of PCR products were carried at Sun biotech Company, Beijing, China.

Sequences generated in this study were analyzed with other sequences obtained from GenBank and recent relevant publications (Santos et al. 2016, Mardones et al. 2017) (Table 1). *Dothidea sambuci* (DAOM231303) was selected as the out group. Sequence data were aligned by MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/index.html>), and edited and visually improved using Bioedit v. 7 (Hall 1999) and MEGA v.5.0 (Tamura et al. 2011). Phylogenetic analyses consisted of maximum likelihood (ML) analysis. A ML analyses was performed using raxmlGUI version 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model. The best scoring tree was selected with a final likelihood value of -

4587.577780 Resulting trees were visualized with TreeView v. 1.6.6 (Page 1996). Sequences generated in this study were deposited in GenBank (Table 1).

## Results

### *Phylogenetic analysis*

One-hundred and ninety-eight taxa, including 37 *Phyllachora*-like taxa, and eight *Polystigma* isolates were included in the phylogenetic analysis. The best scoring RAxML tree is used as the representative tree (Fig. 1).

Three clades (A, B & C) containing members of *Phyllachoraceae* were recognized within the order Phyllachorales (Fig. 1). Clade A comprised most of *Phyllachora* species including three-new species introduced in this study: *P. chloridis*, *P. cynodonticola* and *P. panicicola*. The *P. graminis* strains used in this analysis clustered in different places in Clade A. *Phyllachora graminis* strain TH544 is a sister taxon to the novel species *P. chloridis*. Clade B comprised 13 strains (viz. *Neophyllachora cerradensis*, *N. myrciae*, *N. myrciariae*, *N. subcircinans* and *N. truncatisporum* and *Phyllachora*-like sp.). *Ascovaginospora* sp., *Coccodiella* spp., *Camarotella* spp. and two *Phyllachora*-like species clustered in Clade C. Species of *Polystigma* grouped in three different places.

Three strains of *Po. pusillum* and *Polystigma* sp. (MM163) grouped within the order *Phyllachorales*, while strains of *Po. amygdalinum* and *Po. rubrum* constitute a distinct subclade within the order Xylariales .

## Taxonomy

### *Phyllachoraceae*

*Phyllachoraceae* is a group of host-specific, biotrophic, obligate plant parasites with a tropical distribution and is the largest family within the order Phyllachorales (Santos et al. 2016). Many of the genera of this family have less than ten species and 27 are monotypic (Mardones et al. 2017). *Phyllachora* is the largest genus with 994 species (Wijayawardene et al. 2017, Index Fungorum 2017).

*Phyllachora chloridis* Dayarathne, Shivas. & K.D. Hyde, sp. nov.

Fig. 2

Index Fungorum number: IF552804, Facesoffungi number: FoF02907

Etymology – Epithet derived from host genus, Poaceae.

Holotype – MFLU 15-0173

*Parasitic* on *Chloris* sp. (Poaceae) and unidentified Poaceae sp. *Leaf spots* on host black, abundant, gregarious to scattered, raised, mostly oblong or elongated. Sexual morph: *Pseudostromata* 1–1.2 mm diam., subglobose, scattered, sometimes gregarious, occupying the entire section of the mesophyll, shiny black, with some carbonaceous superficial areas, multilocular. *Ascomata* 80–90 × 90–100 µm diam., perithecia globose, with an almost flat base, lying in leaf tissues and maturing in living leaves, ostiolate, ostiolar canal lacking periphyses. *Peridium* 6–12 µm wide, clypeate, opaque, black, epidermal, thickest adjacent to ostiolar canal, composed of an amorphous layer of host cuticle and epidermal cells, often merging with distorted parenchyma cells infiltrated with fungal hyphae, beneath the clypeus. Lower and lateral peridium composed of two layers; an outer region comprising several layers of dark brown, flattened, thin-walled fungal cells, which merge inwardly with several layers of hyaline, flattened, thin-walled fungal cells. The basal peridium merges outwardly with either a narrow zone of infiltrated and distorted host parenchyma and occasionally lower epidermal cells. *Paraphyses* 0.75–2 µm wide, numerous, persistent, filiform, unbranched, aseptate, slightly longer than asci. *Asci* 50–72 × 6–8µm ( $\bar{x}$  = 62.5 × 7 µm, n = 30), 8-spored, persistent, cylindrical to fusiform, pedicellate, with walls uniform in thickness, not especially thickened at apex, apical ring

inconspicuous. *Ascospores* 8–12 × 3.5–4.8 μm ( $\bar{x}$  = 19.8 × 4 μm, n = 30), 1-seriate, fusiform to oval, hyaline, aseptate, with a central, large guttule and a mucilaginous sheath. Asexual morph: Unidentified

Material examined – THAILAND, Chiang Rai, Doi Mae Salong, on living leaves of *Chloris* sp. (Poaceae), 17 July 2014, R.G. Shivas (MFLU 15-0173, holotype); on living leaves of *Chloris* sp. (Poaceae), 17 July 2014, R.G. Shivas (HKS 97415, isotype). THAILAND, Chiang Mai, Mae Taeng, Pa Pae, Bahn Pa Dheng, 128 Moo 3, Mushroom Research Centre, on living leaves of Poaceae sp. (Poaceae), 22 November 2015, I.D. Goonasekara (MFLU 16-2980).

Note – The main distinguishing characters of *P. chloridis* are the presence of several ascomata within a pseudostroma, occupying the entire section of the mesophyll and 0–1-guttulate, clearly fusiform to oval ascospores. Furthermore, this species is easy to demarcate from the other new species introduced here in having large, centrally guttulate ascospores. *Phyllachora chloridis* is related to *P. graminis* (TH544) but without support. Only two records of *Phyllachora*, *P. cynodontis* and *P. koondrookensis* have been reported from *Chloris* spp. in previous studies (Parbery 1967, 1971). However, *P. chloridis* is clearly distinguished from all the validly documented *Phyllachora* spp. including the above-mentioned species of *Chloris* spp. Hence, we introduce it as a new species. Morphological comparison between *P. chloridis* and related species from *Chloris* spp. are provided in Table 2.

***Phyllachora cynodonticola*** Dayarathne, Goonas. & K.D. Hyde, sp. nov.

Fig. 3

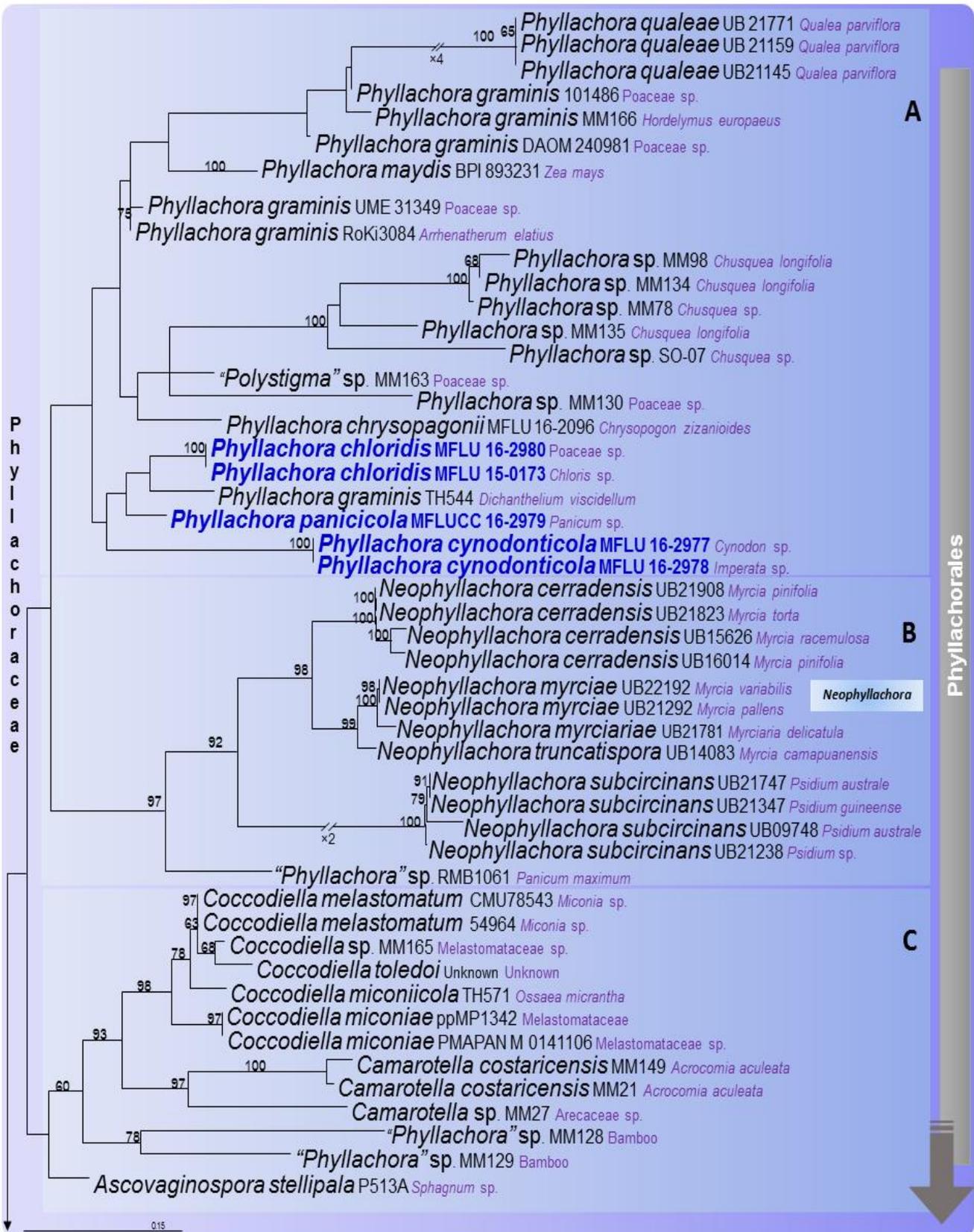
Index Fungorum number: IF552805, Facesoffungi number: FoF02908

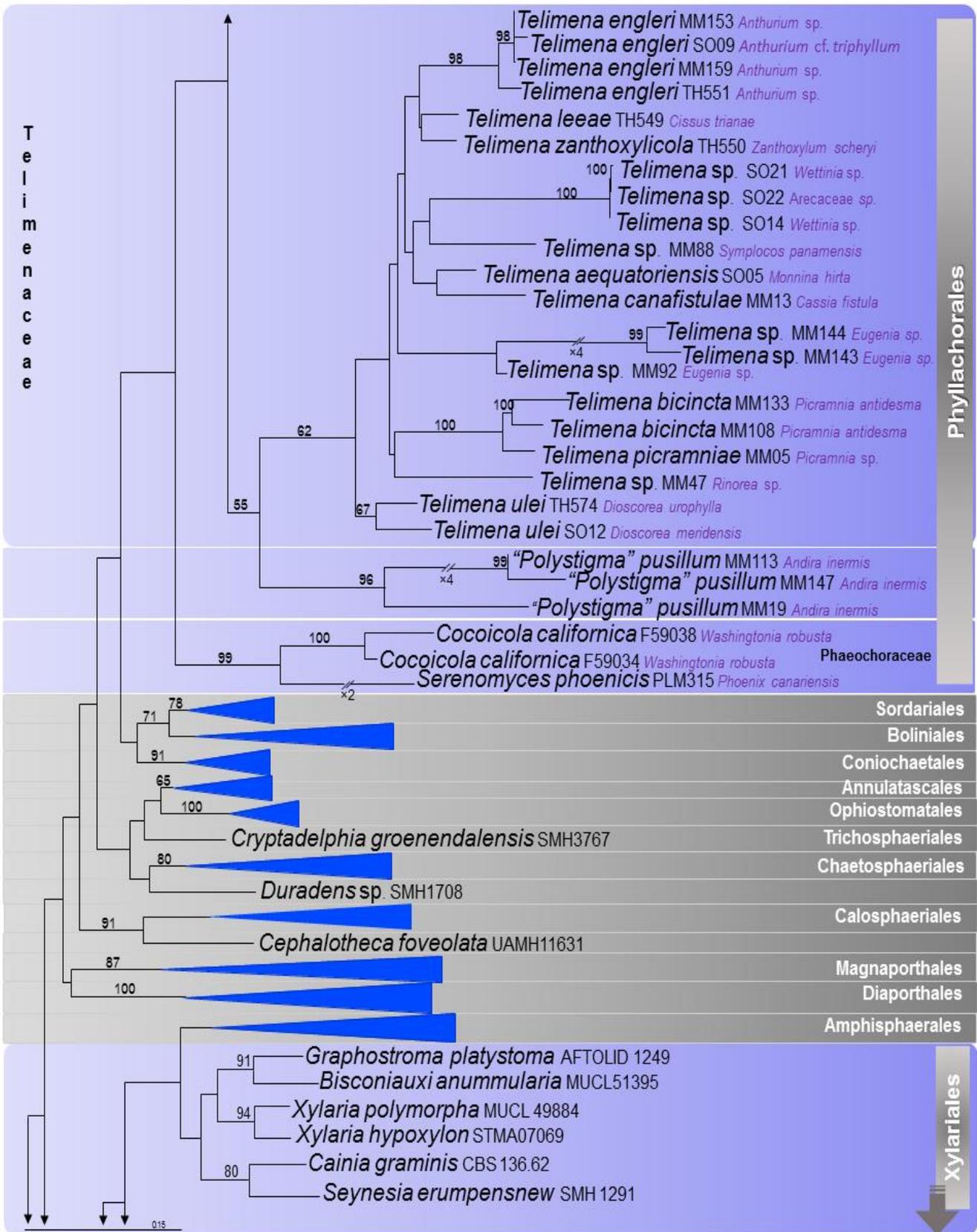
Etymology – Epithet derived from host genus *Cynodon*.

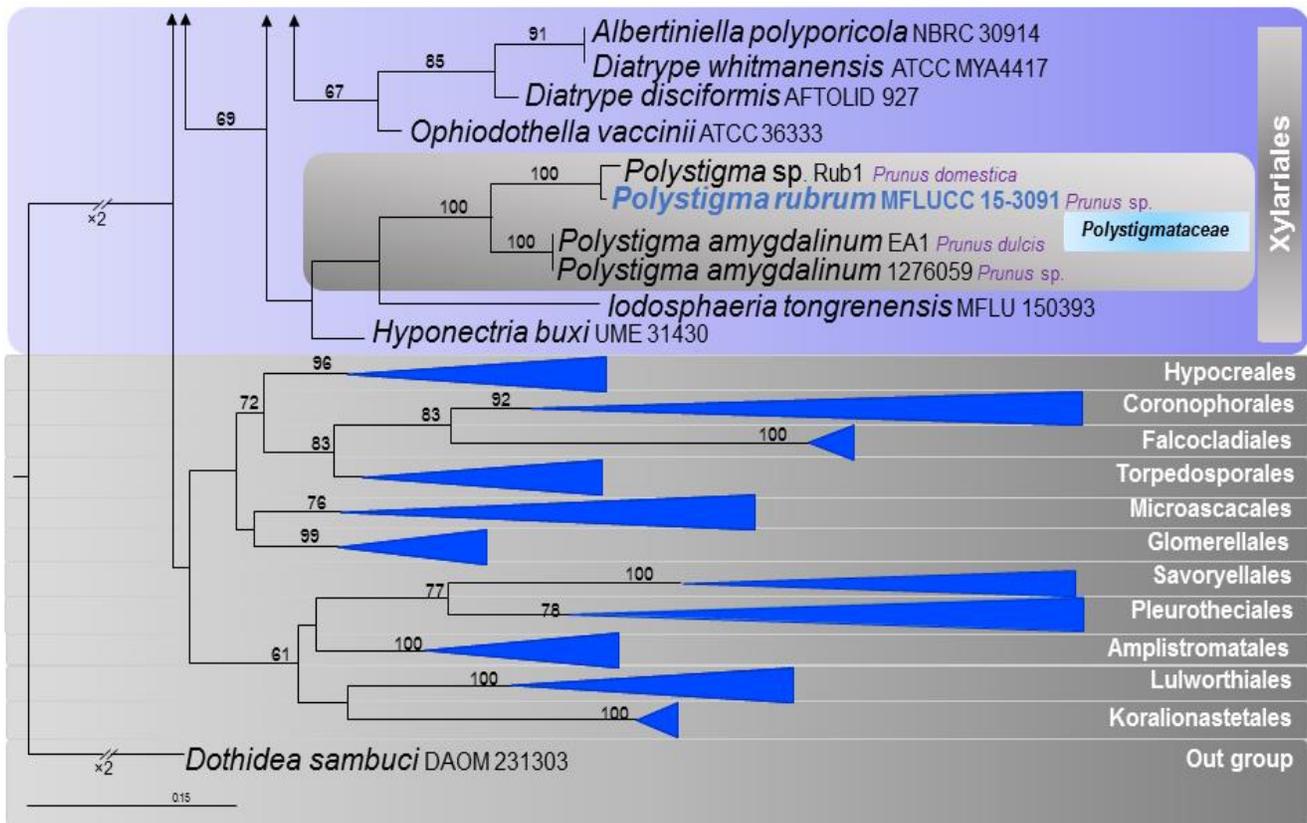
Holotype – MFLU 16-2977

*Parasitic* on Poaceae sp. Sexual morph: *Leaf spots*, 0.6–1 mm wide on host black, abundant, scattered, elongated, parallel with leaf venation, surrounded by yellow necrotic region. *Pseudostromata* 0.7–1 mm wide, subglobose, scattered, sometimes gregarious, occupying the entire leaf section, shiny black, with some carbonaceous superficial areas. *Ascomata* 248–320 μm wide, flattened, globose, with thin walls, lying in leaf tissues and maturing in living leaves, ostiolate, with a clypeus formed by the lateral proliferation of ostiolar hyphae. *Peridium* 28–39 μm, clypeate, composed of black, amorphous layer of host cuticle and epidermal cells, often merging with a lighter pigmented region of yellowish, distorted parenchyma cells infiltrated with fungal hyphae, beneath the clypeus. Lower and lateral peridium composed of two layers; an outer region comprising several layers of black, flattened, thin-walled fungal cells, which merge inwardly with several layers of hyaline, flattened, thin-walled fungal cells. Lateral peridium fuses outwardly with an irregular, narrow region of distorted host parenchyma infiltrated by fungal cells. The basal peridium merges outwardly with either a narrow zone of infiltrated and distorted host parenchyma and or integrates directly with a lower clypeus. *Paraphyses* 1.6–2.4 μm wide, numerous, persistent, filiform, aseptate, branched. *Asci* 48–80 × 16–18 μm ( $\bar{x}$  = 50.5 × 15.6 μm, n = 30), 8-spored, persistent, cylindrical to fusiform, short pedicellate, with walls uniform in thickness, not specially thickened at apex, apical apparatus often present rarely absent, J-. *Ascospores* 10–12 × 5–7 μm ( $\bar{x}$  = 11.5 × 7 μm, n = 30), overlapping 1–2 seriate, fusoid, acute at the ends, with a central concave depression, hyaline, with a mucilaginous sheath. Asexual morph: Unidentified.

Material examined – THAILAND, Chiang Rai, Doi Hang, Amphoe Muang, Mae Korn, roadside near Khun Korn Waterfall, on living leaves of *Cynodon* sp. (Poaceae), 21 January 2015, I.D. Goonasekara (MFLU 16-2977, holotype); on living leaves of *Cynodon* sp. (Poaceae), 21 January 2015, I.D. Goonasekara (HKS 97414, isotype). THAILAND, Chiang Mai, Mae Taeng, Pa Pae, Bahn Pa Dheng, 128 Moo 3, Mushroom Research Centre, on living leaves of *Imperata* sp. (Poaceae), 22 December 2015, I.D. Goonasekara (MFLU 16-2978).







**Figure 1** – Best scoring RAxML tree based on analysis of combined LSU, SSU and ITS sequence data. Maximum likelihood bootstrap values (ML, black)  $\geq 60\%$  are given above the branches. The tree is rooted to *Dothidea sambuci* (DAOM 231303). Newly generated strains are in blue.

Notes – *Phyllachora cynodonticola* differs from other *Phyllachora* species in forming leaf spots surrounded by yellow necrotic regions and 1–2 seriate fusoid, ascospores which are acute at the ends, with a central concave depression and a mucilaginous sheath. *Phyllachora cynodonticola* differs from *P. panicola* in the arrangement of ascospores inside the asci and by having relatively smaller ascospores ( $10\text{--}12 \times 5\text{--}7 \mu\text{m}$  vs  $14\text{--}16 \times 6\text{--}8 \mu\text{m}$ ), whereas, it can be distinguished from *P. chloridis* by having ascospores with a central concave depression. Phylogenetic analysis showed that *P. cynodonticola* has a close affinity to *P. panicola*, but they showed 8% nucleotide differences in the ITS region out of 497 base pairs. *Phyllachora cynodontis* is the only species previously described from *Cynodon* spp. and is clearly different from *P. cynodonticola* mainly from the presence of ovoid ascospores lacking central concave depression and relatively smaller asci ( $20\text{--}25 \times 12\text{--}15$  vs  $48\text{--}80 \times 16\text{--}18$ ) (Table 2). *Phyllachora cynodonticola* and *P. graminis* (type) are also well distinguished morphologically, due to the presence of a centrally located concave depression and a mucilaginous sheath surrounding the ascospores of *P. cynodonticola*, which cannot be observed in *P. graminis*.

***Phyllachora panicola*** Dayarathne & K.D. Hyde, sp. nov.

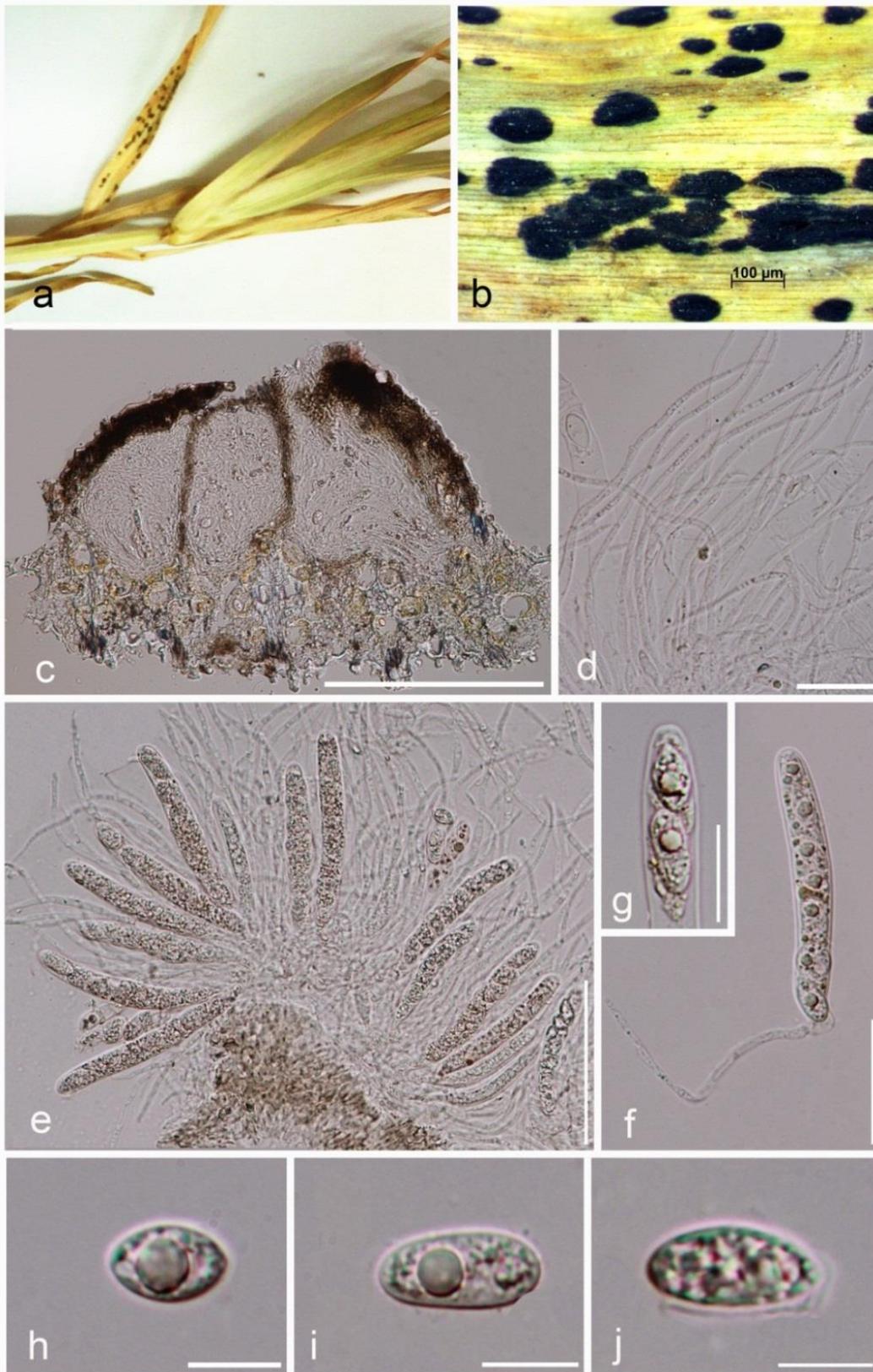
Fig. 4

Index Fungorum number: IF552806, Facesoffungi number: FoF02909

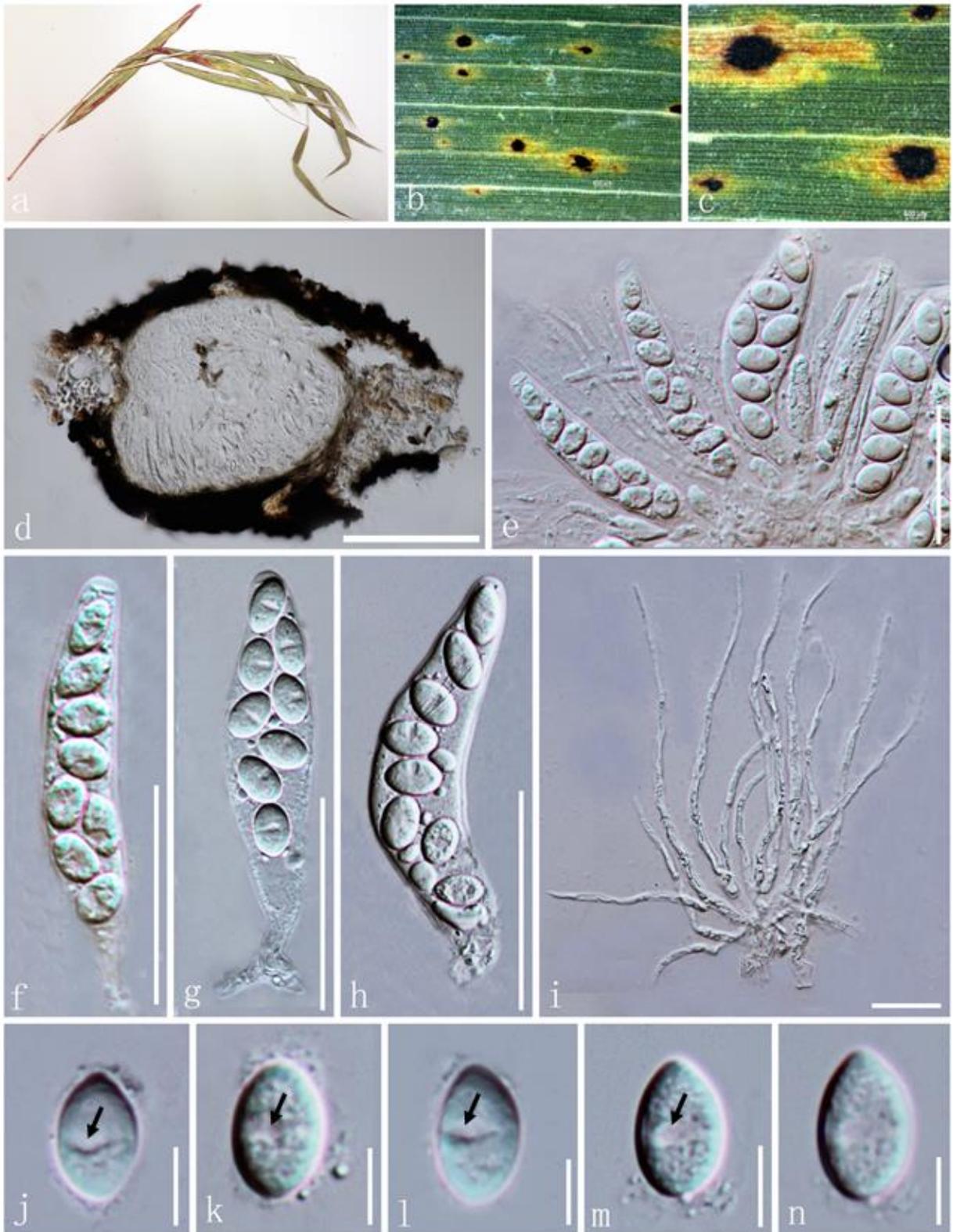
Etymology – Epithet derived from host genus *Panicum*.

Holotype – MFLU 16-2979

*Parasitic* on *Panicum* sp. (Poaceae) Sexual morph: *Leaf spots* on host black, abundant, scattered, raised, oblong or elongated. *Pseudostromata* 0.7–1 mm wide, subglobose, scattered, sometimes gregarious, occupying the entire leaf section, shiny black, with some carbonaceous



**Figure 2** – *Phyllachora chloridis* (MFLU 15-0173 holotype) a,b. Tar spots on living leaves of *Chloris* sp. (Poaceae). c. Section through pseudostroma. d. Paraphyses. e,f. Asci. g. Apex of asci h–j. Ascospores. Scale bars: b = 100 µm, c = 50 µm, e,f = 20 µm, d = 10 µm.



**Figure 3** – *Phyllachora cynodonticola* (MFLU 16-2977 holotype). a. Tar spots on leaves of *Cynodon* sp. b,c. Close up of tar spots on host. d. Section through pseudostroma. e. Asci and paraphyses. f–h. Asci. i. Paraphyses. j–n. Ascospores (arrow indicates the central concave depression). Scale bars: b = 500  $\mu$ m, d = 100  $\mu$ m, e–h = 50  $\mu$ m, i = 10  $\mu$ m, j–n = 5  $\mu$ m.

superficial areas. *Ascomata* 300–500 × 250–300 µm. globose to subglobose, lying in leaf tissues and maturing in living leaves, ostiole inconspicuous. *Peridium* 30–45 µm wide, clypeate, equally thickened, composed of a deeply melanized, black, amorphous layer of host cuticle and epidermal cells, distorted parenchyma cells infiltrated with fungal hyphae, beneath the clypeus, covering the entire pseudostroma. Lower and lateral peridium composed of two layers; an outer region comprising several layers of black, thick-walled fungal cells, which merge inwardly with several layers of hyaline, flattened, thin-walled fungal cells. The basal peridium fuses outwardly with lower clypeus. *Paraphyses* 1–3 µm wide, numerous, persistent, filiform, branched, slightly longer than asci. *Asci* 110–130 × 10–14 µm ( $\bar{x}$  = 118.7 × 12.4 µm, n = 30), 8-spored, persistent, cylindrical to fusiform, pedicellate, with walls uniform in thickness. *Ascospores* 14–16 × 6–8 µm ( $\bar{x}$  = 15.6 × 7 µm, n = 30), overlapping 1-seriate, ellipsoidal, rounded at the ends, with a central concave depression, hyaline, with a thick mucilaginous sheath, 2–3 µm wide. Asexual morph: Unidentified.

Material examined – CHINA, Yunnan Province, Kunming, Botanical garden of Kunming Institute of Botany, on living leaves of *Panicum* sp. (Poaceae), 15 August 2016, M. Dayarathne TAR01 (MFLU 16-2979, holotype); on living leaves of *Panicum* sp. (Poaceae), 15 August 2016, M. Dayarathne TAR01 (HKS 97416, isotype).

Notes – *Phyllachora panicicola* is characterized by the presence of pseudostromata occupying the entire leaf section and oval ascospores with a thick mucilaginous sheath. Phylogenetically this species clearly differs from all the other species used in this analysis. *Phyllachora bonariensis*, *P. mayorii*, *P. microsperma*, *P. minutissima*, *P. panici-proliferi*, *P. paspalicola*, *P. punctum*, *P. stenospora*, *P. tetrasporicola* and *P. urvilleana* are the other species which have been reported from *Panicum* spp. (Parbery 1967, 1971). *Phyllachora panicicola* is most similar to *P. punctum* but can be distinguished by the presence of central concave depression and thick mucilaginous sheath surrounding the ascospores, of the former. A synopsis of *P. panicicola* and other *Phyllachora* species recorded from *Panicum* is provided in Table 2.

### ***Neophyllachora* Dayarathne & K.D. Hyde, gen. nov.**

Index Fungorum number: IF553633, Facesoffungi number: FoF02909

*Parasitic* on Myrtaceae species. Sexual morph: Leaf spots on host black, abundant, scattered, oblong or elongated. *Pseudostromata* subcuticular to intra-epidermal, sometimes occupying the palisade mesophyll layer, circular to irregular or scutiform to hemispherical, gregarious, sparse or coalescent, glabrous, shiny, black, uni to multi-locular (1–12 locules), epiphyllous or amphigenous, often covering most of the leaf surface. *Ascomata* perithecial, ampulliform, immersed in pseudostromatic tissue, ostiolate, clypeate; *ostiole* conspicuous, periphysate; peridium showing layers of compressed melanized cells; clypei well developed, black, hymenium coating the base and the sidewall of the ascomata. *Paraphyses* numerous, filiform or clavate, hyaline, septate, unbranched, thin-walled. *Asci* 8-spored, unitunicate, cylindrical or clavate-fusoid, short-pedicellate, apex obtuse or tapered with a slight thickening, without a conspicuous apical ring. *Ascospores* unicellular, hyaline when immature to light olivaceous at maturity, 1–2-seriate, oblong to ellipsoid or sublunate to lunate, with guttules, surrounded by a gelatinous sheath or with thickened walls at its acute apices. Asexual morph: *Conidiomata* spermatial or conidial, black, immersed in same pseudostromata containing the ascomata. *Spermatial locules* ampulliform, ostiolate; spermatogenous cells hyaline, phialidic, covering internal wall of the conidiomata. *Spermatia* falcate, curved, aseptate, hyaline, with rounded subtruncate ends. Conidiogenous cells holoblastic, lageniform, covering the base of the conidiomata. *Conidia* mostly clavate, ellipsoidal to fusiform or oblong, straight or curved.

Type species – *Neophyllachora myrciae* (Lév.), Dayarathne & K.D. Hyde



**Figure 4** – *Phyllachora panicicola* (MFLU 16-2979 holotype) a. Tar spots on *Panicum* sp. b. Close up of tar spots on host. c. Section through pseudostroma. d. Peridium. e. Paraphyses. f,h. Asci. i-l. Ascospores. Scale bars: c = 100 μm, f-k = 50 μm, d,e = 20 μm,i-l = 10 μm.

**Table 2** Morphological comparison between *P. chloridis*, *P. panicicola* and *P. cynodonticola* with related species reported from Poaceae

Species	Asci ( $\mu\text{m}$ )	Ascospores			Locality	Host	Reference
		Size ( $\mu\text{m}$ )	No. of septa	Shape			
<i>P. bonariensis</i>	45–84 × 9–16	13–17 × 5–7	0–1	Fusoid	Australia, central/ southern Africa, central/ south America, Japan, Java, Nepal, Taiwan, West Indies	<i>Panicum</i> sp.	Parbery 1967, 1971
<i>P. chloridis</i>	50–72 × 6–8	8–12 × 3.5– 4.8	Aseptate	Fusifiform to oval	Thailand	<i>Chloris</i> sp., Poaceae sp.	This study
<i>P. cynodonticola</i>	48–80 × 16–18	10–12 × 5–7	Aseptate	Fusoid, acute at the ends	Thailand	<i>Cynodon</i> sp., <i>Imperata</i> sp.	This study
<i>P. cynodontis</i>	20–25 p12–15 p;	8–15 by 5–6 p.	0–1	Ovoid	World wide	<i>Chloris</i> sp.	Parbery 1967, 1971
<i>P. graminis</i>	80–90 × 8–9.5	9–11 × 6–7	Aseptate	Fusifiform to oval	World wide	<i>Poa</i> sp.	Parbery 1967, 1971
<i>P. mayorii</i>	51–72 × 10–11	10–12 × 4.5	Aseptate	Naviculoid or elongate ovoid	Colombia	<i>Panicum</i> sp.	Parbery 1967,1971
<i>P. microsperma</i>	45 × 7	7–7.5 × 4.5– 5	Aseptate	Oval to broadly ellipsoid	Brazil	<i>Panicum</i> sp.	Parbery 1967,1971
<i>P. minutissima</i>	65–125 × 10–18	17–24 × 7–11	Aseptate	Ovate-acuminate (not extreme) to ovoid or oval	Angola	<i>Panicum</i> sp.	Parbery 1967,1971
<i>P. panicicola</i>	110–130 × 10–14	14–16 × 6–8	Aseptate	Ellipsoidal, rounded at the ends	China	<i>Panicum</i> sp.	This study

**Table 2** Continued.

Species	Asci ( $\mu\text{m}$ )	Ascospores			Locality	Host	Reference
		Size ( $\mu\text{m}$ )	No. of septa	Shape			
<i>P. paspalicola</i>	60–90 × 8–12	10–12 × 6–8	Aseptate	Subglobose to broadly ellipsoid, rarely ovoid	Australia, central and South America, Japan, Papua New Guinea, Philippines, South Africa, West Africa, West Indies	<i>Panicum</i> sp.	Parbery 1967,1971
<i>P. punctum</i>	75–90 × 8–10	9–1 3 × 4– 5.5	Aseptate	Ellipsoid occasionally, oval-ovate	Australia, Brazil, Canada, India, South Africa, United States	<i>Panicum</i> sp.	Parbery 1967,1971
<i>P. stenospora</i>	52–68 × 8–10	7.5–10 × 3.5– 4	Aseptate	Lacriiform	Philippines, Sri-Lanka	<i>Panicum</i> sp.	Parbery 1967
<i>P. tetrasporicola</i>	65–85 by 8–10 p	15–19 by 6-7 p.	Aseptate	Fusiform to ovate- acuminate	Dominican Republic	<i>Panicum</i> sp.	Parbery 1967, 1971
<i>P. urvilleana</i>	60–70 × 7–8	18–20 × 4	Aseptate	Fusiform	Argentina, Buenos Aires, Bahía Blanca; Puerto Argentino	<i>Panicum</i> sp.	<a href="http://www.cybertruffle.org.uk/spgazzini/eng/000192a">http://www.cybertruffle.org.uk/spgazzini/eng/000192a</a>

Notes – Members of *Neophyllachora* are subepidermal, intra-epidermal stromata without a deeper invasion of the mesophyl, and clavate asci; three species appear specifically infecting *Myrcia* species, one on *Myrciaria* species, and one on different *Psidium* species. *Phyllachora myrciae* has characteristically lunate ascospores, and two types of conidia (ellipsoidal and falcate) formed in separate conidiomata, but both showing phialidic conidiogenesis; *P. truncatisporum* on *Myrcia camapuanensis* has sublunate ascospores with both ends showing characteristic wall thickenings; *P. cerradensis* on *M. torta* shows elliptical ascospores with predominantly longer dimensions when compared to *P. subcircinans*, which is found only on *Psidium* species; finally *P. myrciariae* shows ascospores similar in form to those of *P. cerradensis*, but also forms fusoid-clavate phialidic conidia. According to our phylogenetic analysis the species belongs to *Neophyllachora*.

***Neophyllachora myrciae*** (Lév.), Dayarathne & K.D. Hyde, comb. nov.

Index Fungorum number: IF553591

≡ *Catacauma myrciae* (Lév.) Theiss. & Syd., Ann. Mycol. 13(3/4):393. 1915

≡ *Dothideamyrciae* Lév., Ann. Sci. Nat. Bot. 5:264. 1846.

Description – see Santos et al. (2016)

Notes – *Phyllachora myrciae* was described as *Dothidea myrciae* Lév. on *Myrcia* sp. from an undefined location in Brazil (Saccardo 1883). Well-illustrated species were described by Viégas (1948) on the basis of material collected in 1936 from Cerrado on *Eugenia bimarginata*, which is now confirmed as a *Myrcia* species. It has also been reported in 1943 from *M. vestita* (Santos et al. 2016). It is shown to have a broad host spectrum of species, but within the genus *Myrcia* causing tar spots in six different species (*M. decrescens*, *M. dictyophylla*, *M. guianensis*, *M. nivea*, *M. variabilis*, *M. vestita*). All of them produce the same type of pseudostroma, precisely drawn by Viégas (1944), with fusoid asci containing the typically lunate ascospores, and falcate spermatial conidia on a pseudostromatic conidiomata.

***Neophyllachora cerradensis*** (Santos, R.B. Medeiros & Dianese), Dayarathne & K.D. Hyde, comb. nov.

Index Fungorum number: IF553592

≡ *Phyllachora cerradensis* Santos, R.B. Medeiros & Dianese, in Santos, Fonseca-Boiteux, Boiteux, Câmara & Dianese, Mycologia 108(6): 1151 (2016)

Description – see Santos et al. (2016)

Notes – The main characteristics of *N. cerradensis* are the shape of the conidia (mostly clavate), and clearly elliptical ascospores formed within fusoid asci. This species was studied, but not properly described by Medeiros (1994). Later on Santos et al. (2016) properly described this species together with an asexual morph and introduced it as a new species to the genus *Phyllachora* based on morpho-molecular data. According to phylogenetic analysis of combined LSU, SSU and ITS data, all isolates of *N. cerradensis* cluster in a strongly supported subclade (Fig. 1) and share a close relationship to *N. myrciae*, *N. myrciariae*, *N. subcircinans* and *N. truncatispora*.

***Neophyllachora myrciariae*** (Santos & Dianese), Dayarathne, Maharachch. & K.D. Hyde, comb. nov.

Index Fungorum number: IF553593

≡ *Phyllachora myrciariae* Santos & Dianese, Mycologia 108(6): 1151 (2016)

Description – see Santos et al. (2016)

Notes – *Neophyllachora myrciariae* is the first report of a *Phyllachora* species associated with plants belonging to genus *Myrciaria* (Santos et al. 2016). *Neophyllachora myrciariae* can be recognized by intra-epidermal pseudostromata and hyaline, elliptical, irregularly guttulate ascospores covered by a mucoid sheath (Santos et al. 2016). According to our phylogenetic analysis this species formed a well-separated lineage (100% ML) basal to *N. myrciae*.

***Neophyllachora subcircinans*** (Speg.), Dayarathne, Maharachch. & K.D. Hyde, comb. nov.

Index Fungorum number: IF553594

≡ *Phyllachora subcircinans* Speg., Revista Argent. Hist. Nat. 1(6): 413 (1891)

≡ *Catacauma subcircinans* (Speg.) Theiss. & Syd., Annls mycol. 13(3/4): 394 (1915)

Description – see Santos et al. (2016)

Notes – *Neophyllachora subcircinans* comprises subcuticular, partially intraepidermal, epiphyllous, occasionally amphigenous, pseudostromata. Ascospores are hyaline when immature becoming light olivaceous at maturity, guttulate in the middle portion, with irregularly shaped guttules and a thin wall surrounded by a gelatinous sheath. This species was introduced by Spegazzini in 1891 based only on morphological data. Santos et al. (2016) treated this species based on molecular data and grouped in a subclade with *Phyllachora* species parasitic on *Psidium* species and it is closely related to other species within this clade by having subcuticular,

intra-dermal or subepidermal pseudostromata. This is supported by our phylogenetic analysis with combined LSU, SSU and ITS data.

***Neophyllachora truncatispora*** (Viégas), Dayarathne, Maharachch. & K.D. Hyde, comb. nov.

Index Fungorum number: IF55359

≡ *Catacauma truncatisporum* Viégas, *Bragantia* 4(1-6): 145 (1944)

≡ *Phyllachora truncatispora* (Viégas) Bat. & H. Maia, in Batista, Peres & Maia, *Atas Inst.*

*Micol. Univ. Recife* 4: 70 (1967)

Description – see Santos et al. (2016)

Notes – *Neophyllachora truncatispora* is characterized by having sublunate to fusoid, hyaline, smooth-walled, ascospores with characteristic wall thickenings at both acute ends (Viégas 1944). The asexual morph, a stromatic coelomycete comprising filiform, curved, hyaline conidia, tapered on both ends (Santos et al. 2016). In our phylogenetic analysis, it formed a well separated (99% ML) clade basal to a group comprising *N. myrciae*, *N. myrciariae* and *N. subcircinans*.

### Key to species of *Neophyllachora*

1. Parasitic on *Myrcia* or *Myrciaria* species; ascospores ellipsoidal or lunate.....2
1. Parasitic on *Psidium* species; ascospores short-ellipsoidal, oblong to subglobose..*N. subcircinans*
2. Parasitic on *Myrciaria delicatula*; asexual morph with fusoid-clavate conidia.....*N. myrciariae*
2. Parasitic on *Myrcia* species; asexual morph unknown or with filiform conidia.....3
3. Ascospores ellipsoidal, on *Myrcia torta*.....*N. cerradensis*
3. Ascospores typically lunate or lunate-reniform to half-moon shape, on several *Myrcia* species...4
4. Ascospores lunate-reniform to half-moon shape, with acute wall thickenings at both ends; on *Myrcia camapuanensis* ..... *N. truncatispora*
4. Ascospores lunate, without acute wall thickenings at both ends, on *M. decrescens*, *M. dictyophylla*, *M. guianensis*, *M. nivea*, *M. variabilis*, and *M. vestita*.....*N. myrciae*

***Polystigmataceae*** Höhn. ex Nannf., 1932

Facesoffungi number: FoF03518

*Parasitic* on living leaves and shoots of Rosaceae. *Stromata* mostly developing through late spring and summer on living leaves or young shoots, producing conidia in summer and autumn, and ascospores from fallen overwintered leaves the following spring, scattered, slightly raised, irregular, orange or reddish-brown to black, sometimes purplish-black, bordered by apparently healthy leaf tissue. Asexual morph: *Conidial stromata* irregularly-shaped, yellowish-brown in very young lesions, but quickly becoming orange to reddish-brown to black, becoming darker in the central region, hologenous, sometimes distorting the leaves and shoots, the surrounding leaf tissue hardly affected, containing a large number of conidiomata, composed of an upper layer of plant whose cells are filled with bright orange-brown material, an intermediate layer of almost completely occluded angular to vertically elongated fungal cells, and the lower layer similar to the upper one. *Conidiomata* sphaerical, epigenous or hologenous, the ostiole inconspicuous, papillate or apapillate. *Conidiomatal wall* poorly-developed, not clearly distinguishable or composed of hyaline thick-walled cells of *textura angularis*. *Conidiogenous cells* cylindrical, tapering towards the upper region, which are slightly irregular in appearance due to successive conidial scars, usually proliferating sympodially, developing over the entire inner surface of the wall, arising as lateral or terminal branches from short relatively undifferentiated conidiophores. *Conidia* the lower part narrowly lanceolate to fusiform, the upper part filiform, sigmoidally curved, the base truncate, hyaline, aseptate, apparently smooth-walled. Sexual morph: *Ascostromata* irregularly-shaped, usually roughly circular, strongly raising the adaxial surface of the leaf, flat or slightly concave on the abaxial surface, hologenous, reddish-brown to black, sometimes faintly verrucose, the ostioles sometimes inconspicuous, but appearing as small black dots on paler stromata, often somewhat sunken, composed of pigmented outer layers and a hyaline inner layer containing the ascomata.

*Ascomata* sphaerical. *Paraphyses* sparse, gradually tapering towards the apex, very thin-walled, strongly inflated between the septa. *Asci* 8-spored, unitunicate, clavate, long-pedicellate, thin-walled at all stages, the apex obtuse, with an apical ring. *Ascospores* biseriate, cylindrical to ellipsoidal or obovoid, occasionally slightly curved (fabiform), hyaline, aseptate, without a gelatinous sheath (Modified description based on Cannon 1996).

Type genus – *Polystigma* DC., in de Candolle & Lamarck, 1815

Type species – *Polystigma rubrum* (Pers.) DC., in de Candolle & Lamarck, 1815

Notes – The family *Polystigmataceae* is characterized by astromatic or poorly-developed stroma, whose ascus apices do not stain blue with iodine and ascospores without germ pores or slits. Another characteristic feature of the family is the production of appressoria by the germinating ascospores. Most of the species are parasites and cause diseases of economically important plants (Mehrotra & Aneja 1990). Dennis (1968, 1977), Muller & von Arx (1973) and Hawksworth et al. (1983) have included the members of *Phyllachoraceae* within this family and did not recognize *Phyllachoraceae* as a separate family. Hawksworth et al. (1983) have raised it to the ordinal rank, Polystigmatales (Phyllachorales) and included a single family *Phyllachoraceae* with about 23 genera in this order (Mehrotra & Aneja 1990). Since then, species of *Polystigma* were placed within *Phyllachoraceae*. Habibi et al. (2015) reported that *Polystigma amygdalinum*, which was previously placed in Phyllachorales (subclass Sordariomycetidae) was not related to *Phyllachora* species but clustered in the subclass Xylariomycetidae. Our phylogenetic analysis of combined LSU, SSU and ITS data confirms that taxa of *Polystigma* are phylogenetically distant from the Phyllachorales, and belongs to the order Xylariales. Hence, the family *Polystigmataceae* is re-established within the subclass Xylariomycetidae.

***Polystigma* DC., in de Candolle & Lamarck, Fl. franç., Edn 3 (Paris) 6: 164 (1815)**

Facesoffungi number: FoF03519

*Parasitic* on living leaves of Rosaceae. *Stromata* mostly developing through late spring and summer on living leaves or young shoots, producing conidia in summer and autumn, and ascospores from fallen overwintered leaves the following spring, scattered, slightly raised, irregular, orange or reddish-brown to black, sometimes purplish-black, bordered by apparently healthy leaf tissue. Asexual morph: Conidial stromata irregularly-shaped but usually roughly circular, yellowish-brown in very young lesions, but quickly becoming orange to reddish-brown to black, becoming darker in the central region, homogenous, sometimes rolling or crinkling the leaves and shoots, the surrounding leaf tissue hardly affected, containing a large number of conidiomata, composed of an upper layer of plant whose cells are filled with bright orange-brown material, an intermediate layer of almost completely occluded angular to vertically elongated fungal cells, and the lower layer similar to the upper one. Conidiomata sphaerical, epigenous or homogenous, the ostiole epigenous, inconspicuous, slightly papillate or apapillate. Conidiomatal wall poorly - developed, not clearly distinguishable from the stromatal tissue or composed of a narrow layer of small hyaline thick-walled *textura angularis*. Conidiogenous cells cylindrical but gradually tapering towards the upper region, which is slightly irregular in appearance due to successive conidial scars, usually proliferating sympodially, developing over the entire inner surface of the wall, arising as lateral or terminal branches from short relatively undifferentiated conidiophores. Conidia the lower part very narrowly lanceolate to fusiform, the upper part filiform, sigmoidally curved, the base truncate, hyaline, aseptate, apparently smooth-walled. Sexual morph: *Ascstromata* irregularly-shaped, usually roughly circular, strongly raising the adaxial surface of the leaf, flat or slightly concave on the abaxial surface, homogenous, reddish-brown to black, sometimes faintly verrucose, the ostioles sometimes inconspicuous but appearing as small black dots on paler stromata, often somewhat sunken, composed of pigmented outer layers and a hyaline inner layer containing the ascomata. *Ascomata* sphaerical. *Paraphyses* rather sparse, gradually tapering towards the apex, very thin-walled, strongly inflated between the septa. *Asci* 8-spored, unitunicate, clavate, long-pedicellate, thin-walled at all stages, the apex obtuse, with an apical ring. *Ascospores* biseriate,

cylindrical to ellipsoidal, occasionally obovoid, occasionally slightly curved (fabiform), hyaline, aseptate, thin- and smooth-walled, without a gelatinous sheath.

Type species – *Polystigma rubrum* (Pers.) DC., in de Candolle & Lamarck, Fl. franç., Edn 3 (Paris) 5/6: 164 (1815)

***Polystigma rubrum*** (Pers.) DC., in de Candolle & Lamarck, Fl. franç., Edn 3 (Paris) 5/6: 164 (1815) Fig. 5

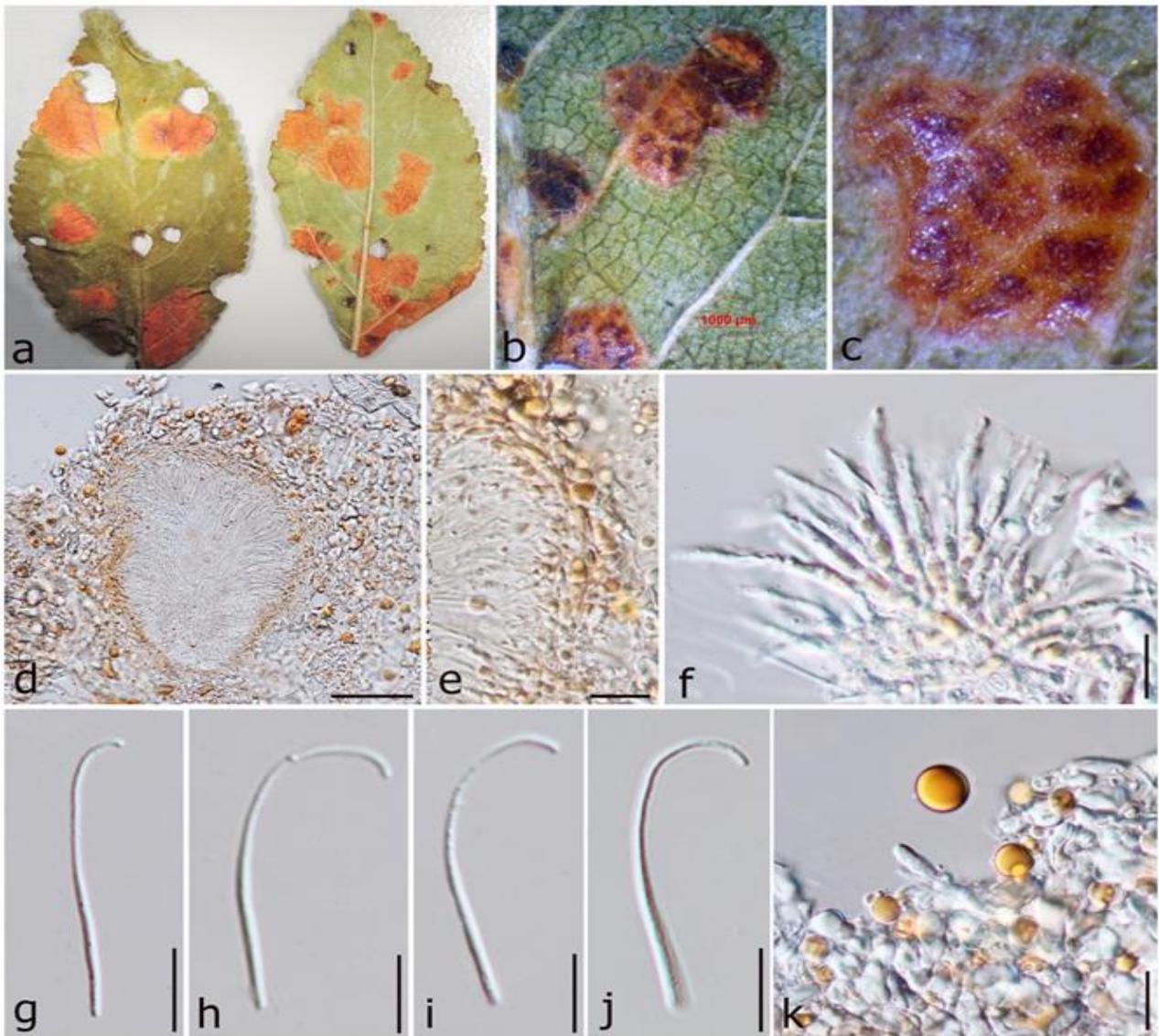
Facesoffungi number: FoF02910

Reference specimen designated here: MFLU 15-3091

Parasitic on *Prunus* spp. *Leaf spots* developing on living leaves, scattered, irregular, swollen, orange to red, bordered by apparently healthy leaf tissue. Asexual morph: Conidial stromata 10–25 mm diam., irregularly-shaped, but usually roughly circular, orangish-brown in very young lesions, but quickly becoming orange to reddish-brown, becoming darker in the central region, locally homogenous (developing within and throughout the leaf tissue), bordered by apparently healthy leaf tissue, with number of conidiomata. Stromatal tissues composed of upper and lower layers of plant tissue 35–50 µm thick whose cells are filled with shiny orange-brown material, and an intermediate layer 250–540 µm thick of almost completely occluded angular to vertically elongated fungal cells. *Conidiomata* 160–280 µm diam., roughly spherical. *Ostiole* inconspicuous. Conidiomatal wall poorly-developed, not clearly distinguishable from the stromatal tissue. Conidiogenous cells developing over the entire inner surface of the wall, often laterally from sequential cells of short conidiophores to 8–12 µm long and 1–2 µm wide; derived from a thin layer of *textura angularis* with hyaline thin-walled cells. Conidiogenous cells 10–30 × 1–3.3 ( $\bar{x} = 25 \times 2.3$  µm, n = 20) µm, at first usually cylindrical but gradually tapering towards apex, which is slightly irregular in appearance due to successive conidial scars, usually proliferating sympodially. Conidia 20–48 × 0.5–0.75 ( $\bar{x} = 35 \times 0.6$  µm, n = 20) µm, the lower part very narrowly lanceolate to fusiform, the upper part filiform, sigmoidally curved to hooked, the base ± truncate, hyaline, aseptate, apparently smooth-walled. Sexual morph: Not observed.

Material examined – RUSSIA, Rostov Region, Shakhty city, Smagin urban microdistrict, abandoned dacha co-operative, allotment garden, on *Prunus cerasifera*, 20 Sept. 2015, T. Bulgakov T 0950 (MFLU 15-3091 reference specimen designated here).

Note – *Polystigma rubrum*, which is common on *Prunus* spp., has been reported mostly from *Prunus cerasifera*, *Pr. domestica*, *Pr. domestica* subsp. *insititia* (syn. *Pr. insititia*), *Prunus spinosa* and *Pr. stepposa* (syn. *Pr. spinosa* ssp. *dasyphylla*). Records on *Pr. salicina* have not been verified, and may refer to sub sp. *ussuriense* (Cannon 1996). This species has a wide distribution throughout the world with abundant collections reported from UK. The typification of *Po. rubrum* accompanies problems if application of the name is to continue with current practice because typification of *Po. rubrum*, introduced by Persoon (Cannon 1996), remains controversial. Cannon (1996) discussed that there are only two collections identified as *Xyloma rubrum* (basionym of *Po. rubrum*) in Persoon's herbarium in L (Nationaal Herbarium Nederland), neither being definite type material as poor collection data are available. L 910263-760 contains two portions of leaves, comprising developing ascomata of *Po. rubrum*. It is inscribed 'Hb. Pers. ded. Letellier' and (in a different hand) '*Dothidea rubra* Fr.'. The material is in poor condition, and its identity is problematic. L 910263-779 contains several leaves with well-developed stromata, but the host is given as *Prunus padus* and two subsequent annotations re-identify the collection as *Polystigma fulvum*. Clearly, neither of these collections is suitable either as a lectotype or a neotype of *Polystigma rubrum* (Cannon 1996). Characteristics of the asexual morph of our isolate is similar with the protologue of *Polystigma rubrum* (Pers.) DC., in de Candolle & Lamarck (Clements & Shear 1931) (Fig. 5). With strong morphological and phylogenetic support we designate this specimen (MFLU 15-3091) as a reference specimen for *Po. rubrum* (sensu Ariyawansa et al. 2014) with the objective of providing the availability of a reliable herbarium material in good condition and corresponding molecular data for future studies.



**Figure 5** – *Polystigma rubrum* (MFLU15-3091 reference specimen) a. Swollen, red/orange/brown leaf spots on *Prunus cerasifera* (Rosaceae). b,c. Close ups of leaf spots on host. d. Section through pycnidial conidioma. e. Conidiomatal wall. f. Conidiogenous cells. g–j. Hooked conidia. k. Red/orange pigments in conidiomatal wall. Scale bars: b = 1000  $\mu$ m, d = 50  $\mu$ m, e = 20  $\mu$ m, f–k = 10  $\mu$ m.

## Discussion

Tar spot diseased specimens from various hosts were subjected to morpho-phylogenetic study and the family *Phyllachoraceae* re-appraised with combined LSU, SSU and ITS sequence data. Three novel species, *Phyllachora chloridis*, *P. cynodonticola* and *P. panicicola*, grouped in family *Phyllachoraceae* within the order *Phyllachorales* in a clade with *Phyllachora* species. *Phyllachora chloridis*, *P. cynodonticola* and *P. panicicola* bear morphological similarities with genus *Phyllachora*. Hence, we introduced them as new species. In addition, the type of *P. graminis* can be easily recognized and differentiated from *P. chloridis*, *P. cynodonticola* and *P. panicicola* by the presence of amphigenous pseudostromata that grows across the entire section of the leaf, occupying the epidermal tissue on both sides of the leaf, with broadly clavate lightly pitted ascospores formed within fusoid asci. The representatives of the type species (*P. graminis*) nested within this clade A, but did not group together (Fig. 1). Sequences generated from 101486 (isolated from Poaceae sp. in Brazil), MM-166 and DAOM 240981 clustered together, while RoKi3084 and UME 31349 formed a separate lineage. Strain TH544 was located sister to the strains of our novel species *P. chloridis*.

However, there are no sequences generated from any type specimens of *P. graminis*. Therefore, it is necessary to obtain sequence data from a specimen of *P. graminis* to determine *Phyllachora sensu stricto*. *Phyllachora furnasensis* has fusoid-clavate asci with fusiform ascospores tapering towards the thick-walled apices, a characteristic that differentiates it from the novel species within this clade (Santos et al. 2016), which have fusiform to oval ascospores with a mucilaginous sheath. *Phyllachora ermidensis* can be clearly recognized as a different species from the species introduced in the present study as they lack a mucilaginous sheath around the ascospores. Santos et al. (2016) showed that *Phyllachora qualeae* which is a parasite on *Qualea multiflora* (Vochysiaceae) is sister to *P. graminis* (UB21307), but distant from most of the *Phyllachora* representatives. In our analysis, it is placed within family *Phyllachoraceae*. However only short ITS sequence data is available for *P. qualeae* strains hence, it is worth to retreat this genus with the multi-gene analyses including probably SSU and LSU data and a greater taxon sampling. *Polystigma* sp. (MM163) reported from *Paspalum* sp. (Poaceae) by Mardones et al. (2017) also clustered with *Phyllachora* species and there are no records to compare the morphology. Therefore, we included it as a phyllachora-like species in our phylogram. All *Phyllachora* species within this group has been reported from species of Poaceae and are host-specific. Two *Phyllachora* strains (MM-128 and MM-139) obtained from Bamboo is phylogenetically apart from *Phyllachora* species and are sister taxa to *Camarotella* spp. within *Phyllachoraceae*. As there are no records of morphology, we kept them as *Phyllachora*-like species during our analysis. It is worth to reconsider their morphology further. They all can be well distinguished as distinct species according to the phylogenetic analysis of combined LSU, SSU and ITS data.

*Phyllachora* representatives with subcuticular to intra-epidermal pseudostromata without a deep penetration into the palisade parenchyma are grouped within the order *Phyllachorales* as a basal clade to most of *Phyllachoraceae* species including the type refs. Characteristics confined to clade B have been well described by Santos et al. (2016). Three well-supported sub-clades were shown within this clade, with two of them comprising species from hosts belonging to the genus *Myrcia*. The other subclade comprises *N. subcircinans*, a parasite on *Psidium* species. *Neophyllachora subcircinans* comprises obclavate asci with oblong, short-ellipsoidal to subglobose ascospores, whereas in *N. cerradensis*, ascospores are ellipsoidal and guttulate. *Neophyllachora myrciariae* forms ascospores similar to those of *N. cerradensis*, but also forms fusoid-clavate phialidic conidia. *Neophyllachora myrciae* produces lunate ascospores inside clavate asci. Apart from these three groups, an additional species with sublunate to fusoid ascospores is located within this clade, *N. trucantispora*, as a basal species to the *N. myrciae* subclade. These observations are well-supported by the studies of Santos et al. (2016). Based on both morphological and phylogenetic evidence, we introduce a novel genus, *Neophyllachora* within *Phyllachoraceae*, to accommodate these taxa. Species of this genus are only found from hosts in the family Myrtaceae. However, most species in this genus have only short ITS sequence data therefore it is recommended to do further analysis with LSU and SSU data. *Phyllachora* sp. (RMB1061) associated with *Panicum maximum* formed a distinct lineage basal to *Neophyllachora* species. There are no morphological records of this species to compare and accommodate in the novel genus.

In a recent study on *Polystigma* by Habibi et al. (2015) *Po. amygdalinum*, which was previously placed in Phyllachorales (subclass Sordariomycetidae) did not group in *Phyllachorales* but within Xylariales. Classification using only few taxonomic characters easily creates polyphyletic taxa that superficially resemble each other (Hausner et al. 1993, Spatafora & Blackwell 1994). Mardones et al. (2017) included *Polystigma amygdalinum* on *Prunus dulcis*, *Po. pusillum* on *Andira inermis* and *Polystigma* sp. on *Paspalum* sp in their analyses; *Polystigma amygdalinum*, did not group among phyllachoraceous fungi but with Trichosphaeriales and Xylariales in the subclass Xylariomycetidae. *Polystigma pusillum* and *Polystigma* sp. cluster with *Phyllachora* species, but not as closely related species in our analysis. Cannon (1991) reported that *Polystigma pusillum* is similar to the genus *Physalospora* (*Hyponectriaceae*) mainly based on similar microscopic features of the two genera. Moreover, its phylogenetic placement within the order is still uncertain. However, based on our results, *Po. amygdalinum* and *Po. rubrum* (type

species) which were isolated from *Prunus* spp. should in any case be placed in the order Xylariales. Hence, the family *Polystigmataceae* is re-instated with *Po. rubrum* as the type species. We could not observe the sexual morph of this species as they occur in spring and our collection was made in the summer. Morphological characters of family *Polystigmataceae* are provided considering all representatives of genus *Polystigma* and a reference specimen for *Po. rubrum* is provided.

*Phyllachora* species have previously been identified based on morphology and there are only a few sequences present in GenBank for comparison. Similarly, most of the species only have ITS data and even most of them are short sequences. This indicates the taxonomical instability still present within family *Phyllachoraceae*. The majority of species belonging to this family has been introduced based on the host on which they occur. Illustrations, original descriptions and molecular data are inadequate for the genus and hence, it is rather controversial when introducing novel species (Parbery 1967, 1971, Gabel et al. 1999, Habibi et al. 2015, Santos et al. 2016, Dayarathne et al. 2016). Furthermore, there are no adequate phylogenetic studies to confirm their host specificities or phylogenetic placements to resolve species confusions in naming. Therefore, it is necessary to synonymize, epitipify or logically remove the controversial older species names to facilitate proper identification of new species within the genus (Dayarathne et al. 2016).

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