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## Tar spot fungi from Thailand

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### Abstract

*Phyllachora* species are responsible for leaf tar spot disease in a wide range of hosts, worldwide. We are studying the phyllachoraceous taxa in northern Thailand. In this paper, we report on two taxa collected from symptomatic graminicolous leaves collected during 2015-2016. The taxa are shown to be novel based on morphological and sequence data and introduced here as *Phyllachora thysanolaenae* collected from *Thysanolaena maxima* and *Phyllachora vetiveriana* from *Chrysopogon zizanioides*. Descriptions, illustrations and molecular data are provided for the new species which are discussed with comparable taxa.

**Key words** – *Phyllachora* – tar spot

### Introduction

Species of Phyllachoraceae are most obligate parasites, producing black “tar spots” on leaves and occasionally stems and fruits of a variety of host plant families with most taxa presumed to be host-specific (Cannon 1997). This family comprises more than a thousand species worldwide, with *Phyllachora* as the type genus (Parbery 1978, Kirk et al. 2008). The genus *Phyllachora* infects more than 1000 host species, and is often found associated with Gramineae, Leguminae, Moraceae and myrtaceous hosts (Cannon 1991, Cannon & Hyde 1999, Pearce & Hyde 2006, Maharachchikumbura et al. 2015, 2016). *Phyllachora* has been reported to be minor plant pathogens, that rarely kill the host tissues (Mehrotra & Aneja 1990, Cannon 1991), but their longevity on host tissue, may attract or provide pathways for secondary infection by other, more severe pathogenic organisms (Cannon 1991, Hock et al. 1992, Parbery 1996). Nonetheless, information on *Phyllachora* species in Thailand is generally lacking, and presently only seven species have been reported, namely *Phyllachora bambusae*, *P. coicis*, *P. cynodontis*, *P. digitariae*, *P. graminis*, *P. pterocarpi* and *P. repens* (Giatgong 1980, Nuangpai et al. 1984, Lenne 1990, Athipunyakom & Likhitekaraj 2009).

There have been few recent studies on *Phyllachora*, mainly because the taxa are biotrophic and will not grow in culture and therefore it is hard to obtain sequence data from fresh collections. Sequence data of nrSSU, nrLSU and ITS showed that Phyllachorales is polyphyletic (Wanderlei-Silva et al. 2003, Habibi et al. 2015). Mardones et al. (2017) introduced a new family

Telimenaceae in Phyllachorales, alongside Phyllachoraceae and Phaeochoraceae based on ribosomal LSU, SSU, ITS, and protein coding RPB2 and TEF1 genes.

In this paper, we introduce two new species of *Phyllachora* discovered during surveys of tar spot disease in northern Thailand from 2015-2016. Descriptions, illustrations and sequence data are provided for the new species which are discussed with comparable taxa.

## Materials & Methods

Survey of plants with tar spot symptoms caused by fungi was carried out from May 2015 through to August 2016. The specimens were collected in Chiang Mai Province, Thailand. Measurements and descriptions of sections of the ascomata, asci and ascospores were carried out by immersing ascomata in water or in Shear's reagent. Unless otherwise stated, all photographs and measurements were made from material mounted in water and 95% lactic acid. Slide were viewed under a Nikon SMZ745T stereo microscope ( $\times 4.5$ ) and ZEISS Scope A1 microscope ( $\times 40-100$ ) where diagnostic morphological features were recorded photomicrographs and measurements were taken.

## DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted directly from DNA extraction kit (E.Z.N.A. Forensic DNA Kit), following the protocols in the manufacturer's instructions (Habibi et. al. 2015). The DNA amplification was obtained by polymerase chain reaction (PCR). nrSSU, nrLSU and ITS region were amplified by the PCR primes reaction mixture and the thermal cycle program applied by (Habibi et. al. 2015). Sequences generated in this study were blasted to search for closet taxa in GenBank database ([www.ncbi.nlm.nih.gov/blast/](http://www.ncbi.nlm.nih.gov/blast/)).

## Results

*Phyllachora thysanolaenae* Tamakaew, Cheew & K.D. Hyde, *sp. nov.*

Mycobank number: MB821879; Facesoffungi number: FoF 03510; GenBank- SSU: MF272147, Fig 1

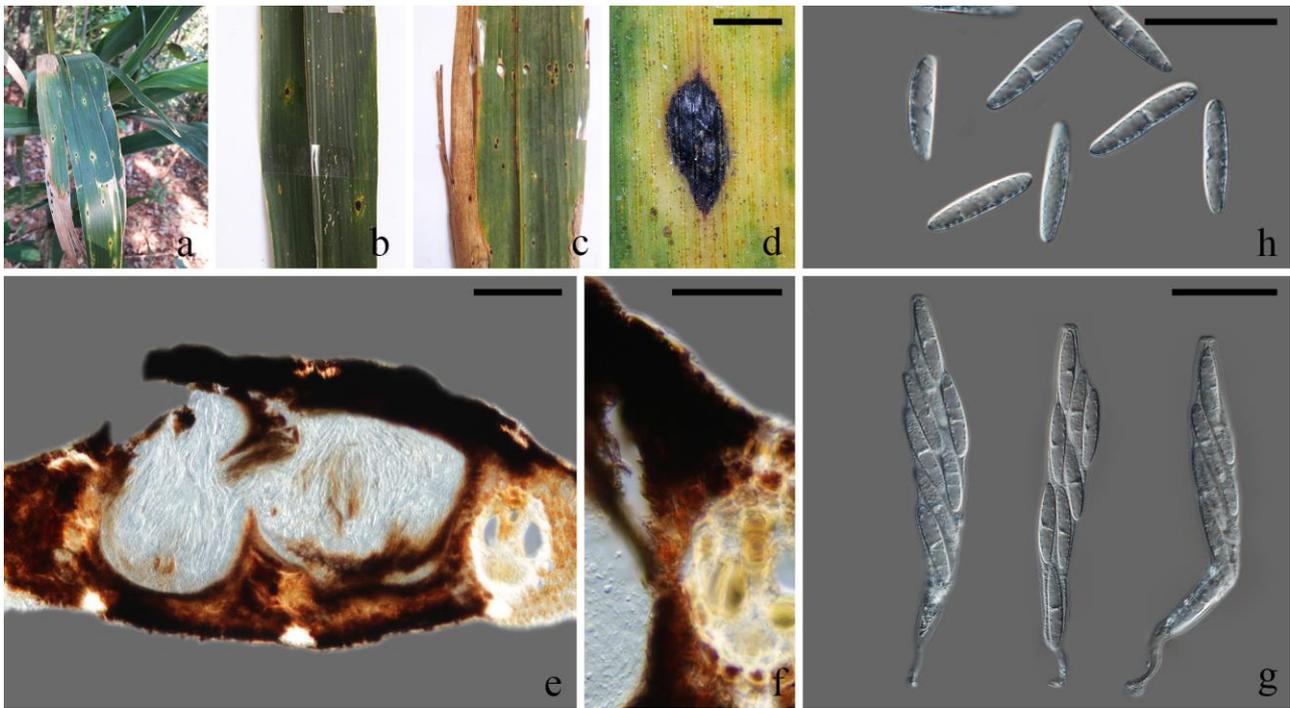
Etymology – Named after its host genus, *Thysanolaena*.

Holotype – MFLU 16-2071

Causing tar spot on living host leaves. Tar spots 0.3–2 $\times$ 0.2–2 mm on upper leaves surface, black punctiform spots, ovoid, shiny, with the raised glossy black clypeus covering the ascomata, chlorotic of reddish yellow discoloured host tissue. **Sexual morph:** *Stromata* uni-multiloculate, 0.3–0.4  $\mu$ m high, 0.6–1.5 mm diam., ovoid, on the upper leaves surface, the stroma immersed with the upper wall of the ascoma, black thick-walled completely occluded by melanins. *Ascomata*, perithecial 175–(205–250)  $\mu$ m high, 270–(300–370)–410  $\mu$ m diam., flask to bowl shape, single or forming in group of 1–4, globose, the ostiole inconspicuous, not or only weakly papillate. *Peridium* composed of thin, dark brown. *Paraphyses* longer than mature asci, filiform, unbranched, septate, not construct at septate. *Asci* 13–18  $\times$  (100–114)–126  $\mu$ m ellipsoid to obclavate, fairly short-stalked, very thin-walled, the apex obtuse to rounded without visible apical structures. *Ascospores* 4–5  $\times$  21–24  $\mu$ m arranged irregularly, cylindrical - fusiform, occasionally very slightly curved, guttulate, thin-walled, hyaline, surrounded by mucilaginous sheath. **Asexual morph:** Not observed.

Material examined – THAILAND, Chiang Mai Province, Chom Thong District, Ban Mae Klang Luang, on living leaves of *Thysanolaena maxima* (Poaceae), 5 March 2016, N. Tamakaew (MFLU 16-2071, **holotype**).

Notes – The collection was identified as belonging to *Phyllachora* on the basis of external symptomatology and morphological characteristics. Of the *Phyllachora* species known from *Thysanolaena maxima* (Parbery & Langdon 1964), *P. thysanolaenae* is distinct from other species in having smaller ascospores. The closest hits using the SSU sequence had the highest similarity to *Phyllachora graminis* isolate UME 31349 (GenBank AF064051, Identities = 962/1039 (93 %))



**Fig. 1** *Phyllachora thysanolaenae* on *Thysanolaena maxima* leaves (holotype, MFLU 16-2071). a, b. Tar spots on upper surface leaves. c. Tar spots on lower surface leaf. d. Close up tar spot lesion. e. Vertical section through tar spot illustrating central ostiole, peridium and position in host tissues. f. Vertical section through peridium. g. Ascospores. h. Asci. Scale bars: d = 5 mm, e = 500  $\mu$ m, f = 30  $\mu$ m, h = 25  $\mu$ m, g = 20  $\mu$ m.

***Phyllachora chrysopogonicola*** Tamakaew, Cheew & Hyde K.D. *sp. nov.*

Mycobank number: MB821880; Facesoffungi number: FoF 03511; GenBank number: LSU: MF372146, ITS: MF372145, Fig 2

Etymology – Named after the host genus from which it was collected, *Chrysopogon*.

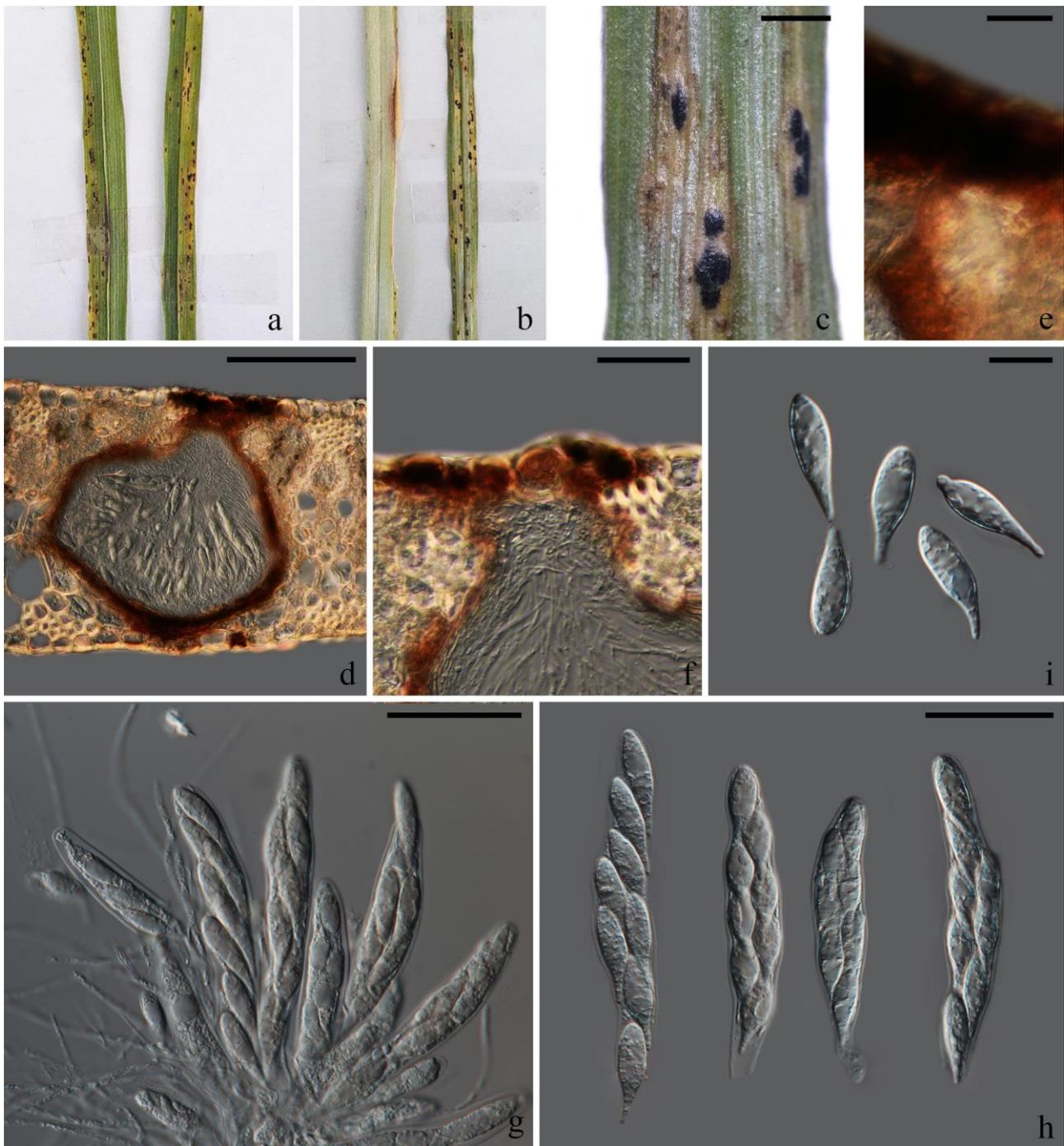
Holotype – MFLU 16-2096

Causing tar spot on living leaves of *Chrysopogon zizanioides*, Tar spots 0.2–0.5×0.1–0.5 mm on upper leaves surface, black punctiform spots, globose to irregular, shiny, low to moderately domed, with the raised glossy black clypeus covering the ascomata. **Sexual morph:** *Stromata* uniloculate, 137–(167–212)–250  $\mu$ m high, 155–(170–210)–245  $\mu$ m diam., globose to irregular, on the upper leaves surface, the stroma immersed with the upper wall of the ascoma, black thick-walled completely occluded by melanins. *Ascomata*, perithecial 115–(145–192)–245  $\mu$ m high, 132–(182–215)  $\mu$ m diam., flask-shaped, bowl-shaped, solitary or forming groups of 1–2, globose, the ostiole inconspicuous, not or only weakly papillate. *Peridium* composed of thin, dark brown. *Paraphyses* filiform, unbranched, septate. Asci, 12.5–15×70–122  $\mu$ m ascospores increase in size and become biseriata. *Ascospores* 6–8×20–(26–30)  $\mu$ m unicellular to papillate to turbinate, clavate, smooth, hyaline, surrounded by mucilaginous sheath. **Asexual morph:** Not observed.

Material examined – THAILAND, Chiang Mai Province, Mae Rim District, Queen Sirikit Botanic Garden, on living leaves of *Chrysopogon zizanioides* (Poaceae), 11 November 2015, N. Tamakaew (MFLU 16-2096, **holotype**).

Notes – *Phyllachora chrysopogonicola* has characters typical of *Phyllachora* in producing black leaf spots, with immersed thin-walled, ostiolate ascomata, and aseptate ascospores (Pearce & Hyde, 2006). Four species have been recorded on *Chrysopogon* spp. namely *P. anthistiriae* (Ahmad, 1969), *P. ischaemi* (Williams & Liu 1976), *P. fallax* (Parbery 1967) and *P. graminis* (Lenne 1990). Compared with morphological characteristics of those known species from *Chrysopogon* spp., *P. chrysopogonicola* is distinguished by having obviously clavate or tear-shaped ascospores. The closest hits using the ITS sequence had the highest similarity to *Phyllachorales* sp. L0002

(GenBank KM108581, Identities = 456/558 (82 %)), and *Phyllachorales* sp. L0001 (GenBank KM108580, Identities = 456/559 (82%)). The closest hits using the LSU sequence had the highest similarity to *Camarops ustulinoides* SMH1988 (GenBank AY346267, Identities = 788/863 (91 %)), and *C. polysperma* Hanson2006-747 (GenBank JN673030, Identities = 785/863 (91%)).



**Fig. 2** *Phyllachora chrysopogonii* on *Chrysopogon zizanioides* leaves (holotype, MFLU 16-2096,). a, b. Tar spots on upper surface. c. Close up of tar spots on leaf. d. Vertical section through tar spot illustrating central ostiole, peridium and position in host tissues. e. Vertical section through peridium. f. Vertical section through ostiolar canal. g. Asci and paraphyses h. Asci, i. Ascospores. Scale bars: c = 5 mm, d = 100  $\mu$ m, e = 30  $\mu$ m, f = 20  $\mu$ m, g = 30  $\mu$ m h. = 30  $\mu$ m, I = 20  $\mu$ m.

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