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Fungi from Asian Karst formations I. *Pestalotiopsis photinicola* sp. nov., causing leaf spots of *Photinia serrulata*

Chen YY[1,2,3], Maharachchikumbura SSN[4], Liu JK[3,5], Hyde KD[6], Nanayakkara RR[3], Zhu GS[2], Liu ZY[3*]

[1]*Agricultural College of Guizhou University, Guiyang, 550006, P.R. China*
[2]*Guizhou Institute of Crop Genetic Resources, Guiyang, 550006, P.R. China*
[3]*Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang 550006, P. R. China*
[4]*Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 8, 123 Al Khoud, Oman*
[5]*Guizhou Institute of Biotechnology, Guiyang, 550006, P.R. China.*
[6]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100. Thailamd.*

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Abstract

This is the first in a series of papers on the fungi growing on plants in the Karst formations of the Asian region. In this study, we collected leaf specimens of *Photinia* with numerous spots from Guiyang Botanical Gardens, Guiyang, China. A combination of morphological characters, together with analyses of combined ITS, TUB and TEF sequence data, distinguished the taxon as a new species of *Pestalotiopsis*. The new taxon is herein described as *Pestalotiopsis photinicola* and discussed in relation to the most related taxa.

Key words – Karst fungi–new species–phylogeny–taxonomy

Introduction

Karst topography is a landscape formed from the dissolution of soluble rocks such as limestone, dolomite, and gypsum and is characterized by underground drainage systems with sinkholes, dolines and caves (Gorbushina et al. 2003). Rainfall and temperatures vary greatly and the formations can be regarded as extreme habitats, especially when soils are sparse. The south China Karst system represents three of the world's most spectacular examples of humid tropical to sub-tropical karst landscapes and extends over a surface of 500,000 square kilometers and lies mainly in the provinces of Guizhou, Yunnan and Guangxi (World Wildlife Fund 2016). The Karst formations are also found in Cambodia, Laos, Malaysia, Myanmar, Thailand and Vietnam (Gunn 2004). Guizhou Province is located in the center of the Southeast Asian Karst Region where it has the most developed karstification, and most diverse karst types, which is also the largest karst formation in the world (Han & Liu 2004). This area has richest biodiversity; there are more than 6000 higher plant species, which include *Cathaya argyrophyll*, *Cycas guizhouensis*, *Davidia involucrata* and *Taxus chinensis*, threatened and endemic species (http://www.worldwildlife.org/ecoregions/pa0101).

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Corresponding author: ZouYi Liu – gzliuzuoyi@163.com

The genus *Pestalotiopsis* Steyaert (1949) is an appendage-bearing, conidial, asexual taxon (coelomycetes) in the family Pestalotiopsidaceae (Hu et al. 2007, Maharachchikumbura et al. 2012, 2015, 2016, Senanayake et al. 2015), and is common in tropical and temperate ecosystems (Maharachchikumbura et al. 2014). Species of *Pestalotiopsis* cause a variety of diseases and are often isolated as endophytes or saprobes plants (Zhang et al. 2012 a, b, 2013, Maharachchikumbura et al. 2013 a, b, c). *Pestalotiopsis* consists of around 300 names (Index Fungorum 2017) and there are various reports that *Pestalotiopsis* species produce a diverse array of chemical compounds (Xu et al. 2010, Maharachchikumbura et al. 2011).

We are carrying out a survey of diseases of native, ornamental and medicinal plants in the Karst regions of Asia. The aim of this paper is to introduce a new species of *Pestalotiopsis* from *Photinia serrulata*, which is a very popular ornamental shrub in China and it is commonly known as Chinese Photinia. The taxon associated with the disease was isolated into culture, where it also produced a *Pestalotiopsis* asexual morph. Molecular and morphological analysis showed this fungus to be a new species of *Pestalotiopsis* and it is described here as *Pestalotiopsis photinicola*.

Materials & Methods

Isolation and morphological studies

Fresh specimens were obtained from leaf spots on living leaves of *Photinia serrulata* collected from Guiyang Botanical Gardens, Guiyang Province, China. To induce sporulation, infected leaves were placed in sterilized Petri-dishes with moistened sterile filter paper. The Pestalotiopsis species present on the samples was isolated from single spores using the method of Chomnunti et al. (2014). The pure cultures were incubated at room temperature for 2–5 days and sub-cultured onto fresh PDA. Colony colour on PDA was determined with the colour charts of Rayner (1970). Observations were made using a Motic SMZ-168 Series stereomicroscope and photographed by a Nikon E80i microscope-camera system. Measurements were made with the Tarosoft (R) Image Frame Work (Liu et al. 2010). Facesoffungi numbers are provided as detailed in Jayasiri et al. (2015). Type materials are deposited at the herbarium of Guizhou Academy of Agriculture Sciences (GZAAS). Fungi isolated in our study were deposited at Guizhou Culture Collection, Guiyang, China (GZCC).

DNA extraction and PCR condition and phylogenetic analysis

Total genomic DNA was extracted from fresh fungal mycelia (500 mg) scraped from the margin of a colony on a PDA plate incubated at 25 °C for 7–10 days. The ITS and 5.8S region of rDNA molecule was amplified using primer pairs ITS4 and ITS5 (White et al. 1990), β-tubulin gene region was amplified with primer pairs BT2A and BT2B (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997) and tef1 was amplified using the primer pairs EF1–526F and EF1–1567R (Rehner 2001). The thermal cycling programs are followed in Maharachchikumbura et al. (2012). Sequencing of the PCR amplicons was conducted using the same primers as those used for the amplification reactions. The PCR products were verified by staining with Goldview (Guangzhou Geneshun Biotech, China) on 1 % agarose electrophoresis gels. DNASTAR Lasergene SeqMan Pro v.8.1.3 was used to obtain consensus sequences from sequences generated from forward and reverse primers and these were subsequently lodged with GenBank (Table 1).

Most of the taxa used in this study are derived from Maharachchikumbura et al. (2012, 2013a,b,c, 2014) and Zhang et al. (2012a,b, 2013). Multiple sequence alignments were generated with MAFFT v.7 (http://mafft. cbrc.jp/alignment/server/ index.html); the alignment was visually improved with MEGA v.5.2.2 (Kumar et al. 2012). Phylogenetic analyses of the sequence data consisted of maximum likelihood (ML) analyses of the combined aligned dataset using raxmlGUI v.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 resulting trees were printed with FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and the layout was made with Adobe Illustrator CS v.6.

Table 1 GenBank Accession numbers of the sequences used in phylogenetic analysis

Species	Strain No.	GenBank Accession Numbers.		
		ITS	TUB	TEF
Pestalotiopsis adusta	ICMP 6088	JX399006	JX399037	JX399070
P. australis	CBS 114193	KM199332	KM199383	KM199475
P. arceuthobii	CBS 434.65	KM199341	KM199427	KM199516
P. arengae	CBS 331.92	KM199340	KM199426	KM199515
P. australasiae	CBS 114126	KM199297	KM199409	KM199499
P. biciliata	CBS 790.68	KM199305	KM199400	KM199507
P. biciliata	CBS 124463	KM199308	KM199399	KM199505
P. biciliata	CBS 236.38	KM199309	KM199401	KM199506
P. brassicae	CBS 170 26	KM199379	----	KM199558
P.camelliae	MFLUCC 12-0277	JX399010	JX399041	JX399074
P. clavata	MFLUCC 12-0268	JX398990	JX399025	JX399056
P. chamaeropis	CBS 186.71	KM199326	KM199391	KM199473
P. colombiensis	CBS 118553	KM199307	KM199421	KM199488
P. digitalis	ICMP 5434	KP781879	KP781883	
P. diploclisiae	CBS 115587	KM199302	KM199419	KM199486
P.diversiseta	MFLUCC 12-0287	JX399009	JX399040	JX399073
P. dracontomelon	MFUCC 10-0149	KP781877		KP781880
P. ericacearum	IFRDCC 2439	KC537807	KC537821	KC537814
P. furcata	MFLUCC 12-0054	JQ683724	JQ683708	JQ683740
P. gaultheria	IFRD 411-014	KC537805	KC537819	KC537812
P. grevilleae	CBS 114127	KM199300	KM199407	KM199504
P. hawaiiensis	CBS 114491	KM199339	KM199428	KM199514
P. hollandica	CBS 265.33	KM199328	KM199388	KM199481
P. humus	CBS 336.97	KM199317	KM199420	KM199484
P. inflexa	MFLUCC 12-0270	JX399008	JX399039	JX399072
P. intermedia	MFLUCC 12-0259	JX398993	JX399028	JX399059
P. intermedia	MFLUCC 12-0259	JX398993	JX399028	JX399059
P. italiana	MFLUCC 12-0657	KP781878	KP781882	KP781881
P. jesteri	CBS 109350	KM199380	KM199468	KM199554
P. kenyana	CBS 442.67	KM199302	KM199395	KM199502
P. knightiae	CBS 114138	KM199310	KM199408	KM199497
P. licualacola	HGUP 4057	KC436006	KC481683	KC481684
P. linearis	MFLUCC 12-0271	JX398992	JX399027	JX399058
P. malayana	CBS 102220	KM199306	KM199411	KM199482
P. monochaeta	CBS 144.97	KM199327	KM199386	KM199479
P. novae-hollandiae	CBS 130973	KM199337	KM199425	KM199511
P. oryzae	CBS 353.69	KM199299	KM199398	KM199496
P. papuana	CBS 331.96	KM199321	KM199413	KM199491
P. parva	CBS 265.37	KM199312	KM199404	KM199508
P. photinicola	GZCC 16-0028	KY092404	KY047663	KY047662
P. portugalica	CBS 393.48	KM199335	KM199422	KM199510
P. rhodomyrtus	HGUP 4230	KF412648	KF412642	KF412645
P. rosea	MFLUCC 12-0258	JX399005	JX399036	JX399069
P. scoparia	CBS 176.25	KM199330	KM199393	KM199478
P. shorea	MFLUCC 12-0314	KJ503811	KJ503814	KJ503817
P. spathulata	CBS 356.86	KM199338	KM199423	KM199513
P. trachicarpicola	OP068	JQ845947	JQ845945	JQ845946
P. telopeae	CBS 114161	KM199296	KM199403	KM199500
P.unicolor	MFLUCC 12-0276	JX398999	JX399030	---
P. verruculosa	MFLUCC 12-0274	JX398996	----	JX399061
Neopestalotiopsis saprophyta	NN047136	JX398982	JX399017	JX399048

Results

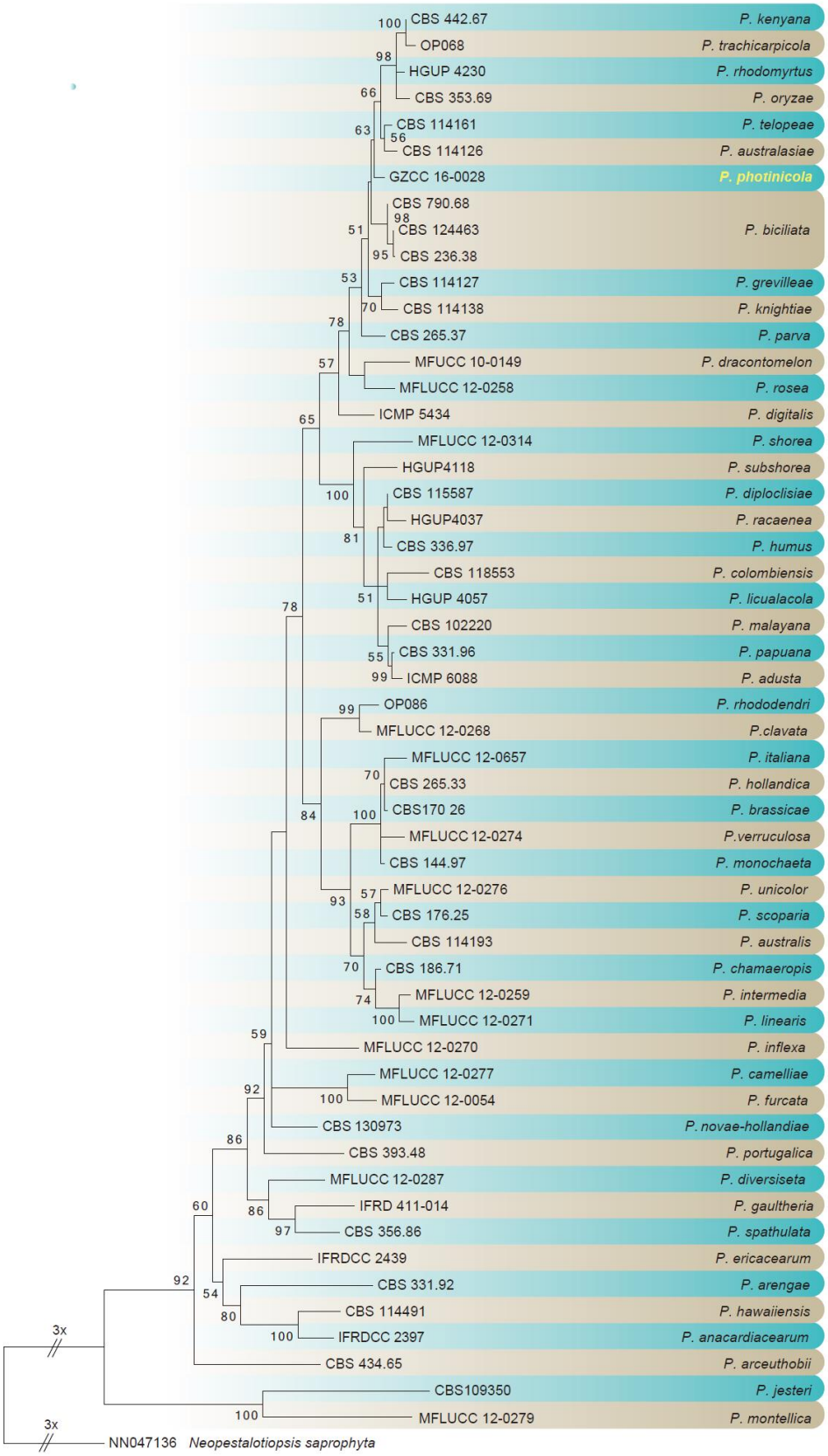

Fig 1. Maximum likelihood (ML) majority rule consensus tree for the analyzed *Pestalotiopsis* isolates. RAxML bootstrap support values (ML > 50%) are given at the nodes and new isolates are in yellow. The scale bar represents the expected number of changes per site. The tree was rooted to *Neopestalotiopsis saprophytica*.

Phylogenetic analyses

The aligned data matrix for combined ITS, β-tubulin and tef1 datasets consisted of 55 sequences representing 34 isolates of *Pestalotiopsis*, with *Neopestalotiopsis saprophyta* as the out-group taxon, and consisted of 1,633 total characters, including gaps. Our phylogenetic analyses showed that the isolate of *P. photinicola* (GZCC 16-0028) formed a clade with moderate statistical support (60), and show close phylogenetic relationship with *P. telopeae*, *P. australasiae* and *P. biciliata*, but formed a distinct clade within the genus and herein we introduce it as a new species *P. photinicola*.

Taxonomy

Pestalotiopsis photinicola Y.Y. Chen, K.D. Hyde, J.K. Liu; & Maharachch., *sp. nov.*

Index Fungorum number: IF552562, Facesoffungi number: FoF 02914, Fig 2

Etymology – named after the host from which the holotype was isolated

Holotype: GZAAS 16-0019

Associated with leaf spots of *Photinia serrulata*. Asexual morph: *Conidiomata*, brown, epidermal to subepidermal, separate or confluent, dehiscence irregular, 150–270 μm diameter ($\overline{x}$ = 215 μm, n = 20). *Conidiophores* most often reduced to conidiogenous cells, simple or branched, hyaline, smooth-walled. *Conidiogenous cells* discrete, hyaline, 1-celled, branched or separate at the base, formed from the upper cells of the pseudoparenchyma, collarette present and not flared. *Conidia* 18–24 × 4–5 μm ($\overline{x}$ = 21.5 μm, n = 20), fusiform, straight to slightly curved, 4-septate; basal cell obconic, hyaline, thin- and smooth-walled, 5.5–8 μm long ($\overline{x}$ = 6.6 μm, n = 10); three median cells, concolourous, brown, septa and periclinal walls darker than rest of the cell, 11–15 μm long ($\overline{x}$ = 13 μm, n = 10); second cell from base 4.5–5 μm long ($\overline{x}$ = 4.7 μm, n = 10); third cell 4.5–5.5μm long ($\overline{x}$ =5 μm, n=10); fourth cell 5–5.5 μm long ($\overline{x}$ = 5.2 μm, n = 10); apical cell, hyaline, conic to subcylindrical, 3–5 μm long ($\overline{x}$ = 4 μm); 1–3 tubular apical appendages (mostly 3), arising from the apex, 12–22 μm long ($\overline{x}$ = 17.5 μm); basal appendage single, tubular, un-branched, centric, 4–8 μm long ($\overline{x}$ = 6 μm). Sexual morph: Undetermined.

Culture characteristics – Colonies attaining 40–50 mm diameter after 7 days at 25 °C on PDA, edge irregular, white, dense aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture yellow-orange.

Material examined – CHINA. Guizhou Province, Guiyang Botanical Gardens, on leaves of *Photinia serrulata*, October 2015. Y.Y. Chen (GZAAS 16-0019; **holotype**), ex-type living culture GZCC 16-0028.

Discussion

In this study, one new species, *Pestalotiopsis photinicola* from disease leaves of *Photinia serrulata* from Guizhou Province, China is characterized in terms of morphology and phylogeny.

Pestalotiopsis photinicola is morphologically (Fig. 2) and phylogenetically (Fig. 1) distinct from other species in the genus. It is morphologically similar to *P. australasiae* (24.5–29 × 6.5–8 μm), *P. biciliata* (22–28.5 × 6–7.5 μm) and *P. telopeae* (24.5–31 × 6–8 μm) (Maharachchikumbura et al. 2014). However, *Pestalotiopsis photinicola* can clearly be distinguished from these species by its smaller conidia (18–24 × 4–5 μm). *Pestalotiopsis photinicola* is morphologically most similar to *P. chamaeropis* (Maharachchikumbura et al., 2014); however, the molecular analysis showed that they are phylogenetically distinct (Fig. 1).

Species of *Pestalotiopsis* are important as phytopathogens (Maharachchikumbura et al. 2012) and many species have been named according to their host association (Maharachchikumbura et al. 2011) and only a small number of characters are available to distinguish them reliably (Maharachchikumbura et al. 2013). Use of ITS sequences alone does not resolve pestalotiopsis-like taxa well, however, combined gene (ITS, *β-tubulin and tef1*) analyses have resolved species successfully (Maharachchikumbura et al. 2012) and it works for the present study.

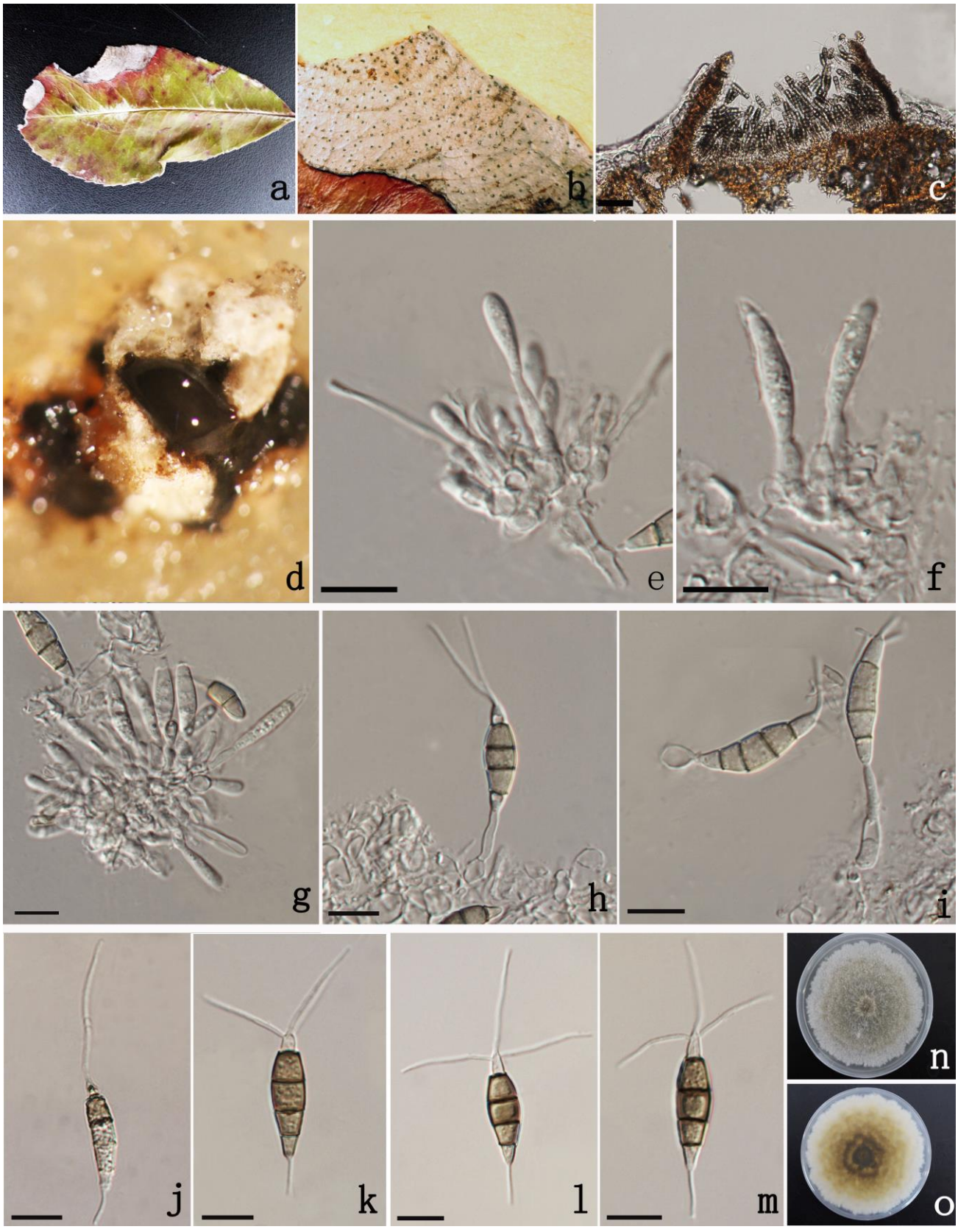

Fig. 2 *Pestalotiopsis photinicola* (holotype, GZAAS 16-0019). a, b. Leaf spot on living leaves of *Photinia serrulata*. c. Section of conidioma. d. Conidiomata on PDA. e–i. Conidiogenous cells and developing conidia. j–m. Conidia. Scale bars: c = 50 μm, e-m = 10 μm

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