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ORIGINAL ARTICLE

Species diversity of *Pseudocercospora* from Far East Asia

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Abstract This study reflects on the monophyly of, and species diversity within, the genus *Pseudocercospora* in Far East Asia. Morphological characteristics and phylogenetic analyses of *Pseudocercospora* species were based on type specimens and ex-type cultures, which were collected from Japan and Taiwan. A phylogenetic tree was generated from multilocus DNA sequence data of the internal transcribed spacer regions of the nrDNA cistron (ITS), partial actin (*actA*), and partial translation elongation factor 1-alpha (*tef1*), as well as the partial DNA-directed RNA polymerase II second largest subunit (*rpb2*). Based on these results, *Pseudocercospora amelanchieris* on *Amelanchier* and *Ps. iwakiensis* on *Ilex* were newly described from Japan, and a further 22 types (incl. two neo-, five lecto-, and 15 epitypes), were designated. The genus *Pseudocercospora* as presently circumscribed was found to be monophyletic, while the secondary barcodes, *actA*, *tef1*, and *rpb2* were shown to be well suited to delimitate species within the genus.

Keywords Epitypification · New species · Phylogenetic relationship · Species criteria · Taxonomy

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Introduction

The genus *Pseudocercospora* Spegazzini (1910) is a well-known genus of cercosporoid hyphomycetous fungi that contains numerous important plant pathogenic species. Originally, the sexual morph of *Pseudocercospora* was accommodated in *Mycosphaerella* (Mycosphaerellaceae, Capnodiales), along with approximately 30-odd other asexual genera (Crous 2009), most of which are now recognized as distinct genera within the Mycosphaerellaceae. In the original monographic treatment of this generic complex published by Chupp (1954), almost all species were placed in the genus *Cercospora*. Since then, numerous species of *Cercospora* have been reassigned to *Pseudocercospora* based on a combination of morphological characteristics such as conidial pigmentation, and the structure of conidiogenous loci (scars) and hila (Deighton 1976, 1979, 1983, 1987, 1990). The generic concept was refined in subsequent studies (Pons and Sutton 1988; Braun 1995; Crous and Braun 2003; Crous et al. 2013), and the genus *Pseudocercospora* is now widely accepted among mycologists and plant pathologists. Presently, the taxonomic criteria for distinguishing genera and species of cercosporoid fungi, including *Pseudocercospora*, are sequentially published in a series by Braun et al. (2013, 2014, 2015a, b, 2016a). Based on several recent phylogenetic studies, *Mycosphaerella* has been reduced to synonymy under *Ramularia* spp. (Videira et al. 2015, 2016), while the sexual morphs of other cercosporoid genera have been regarded as

"mycosphaerella-like" and named under their asexual generic names (Guatimosim et al. 2016). A single generic name is now used for species of *Pseudocercospora* (Wingfield et al. 2012; Crous et al. 2013) under the International Code of Nomenclature for algae, fungi, and plants (ICN) (McNeill et al. 2012).

Regional monographic studies of *Pseudocercospora* species in East Asia began with descriptions of *Cercospora* spp. in the "Descriptive Catalogue of Formosan Fungi vol. 1–11 (1920–1959)" by Sawada in Taiwan. Thereafter, many researchers have published regional monographs from Asian countries based on the criteria for *Cercospora* sensu lato by Chupp (1954) or the comprehensive criteria by Deighton (1976 and subsequent papers). Monographs from the following countries have been published: Japan (Katsuki 1965; Nakashima et al. 2011), Singapore and the Malay Peninsula (Yen and Lim 1980), Taiwan (Hsieh and Goh 1990; Kirschner 2013; Kirschner and Liu 2014), China (Guo and Hsieh 1995), Korea (Shin and Kim 2001; Crous et al. 2013), and Thailand and Laos (Phengsintham et al. 2013a, b). In many cases, voucher specimens of the recorded species, including types and their derived isolates are missing, cultures were never deposited, or the use thereof is restricted for various reasons.

A phylogenetic analysis of *Pseudocercospora* using multilocus sequence data was performed by Crous et al. (2013). From these findings, it was evident that *Pseudocercospora* species have a host range that is chiefly restricted to a single plant genus, with a few exceptions. Phylogenetic analyses based on the large subunit of the nuclear ribosomal RNA (nrRNA) gene (LSU; 28S) sequence data and the concatenated multi-locus sequence data, composed of partial gene sequences of the internal transcribed spacers and intervening 5.8S nuclear nrRNA (ITS), partial actin (*actA*), and translation elongation factor 1-alpha (*tef1*) regions, allowed for the delimitation of most species, including the classification of disjunct species across continents, that may be misidentified based on morphological identifications made without the examination of type materials. In addition, the phylogenetic backbone based on concatenated multi-locus sequence data has also been used to understand the regional diversity of *Pseudocercospora* spp. (Bakhshi et al. 2014; Shivas et al. 2015; Guatimosim et al. 2016; Silva et al. 2016), as well as to identify plant pathogenic species (Parreira et al. 2014; Crous et al. 2015; Park et al. 2015; Liang et al. 2016). However, those studies also revealed *Pseudocercospora* to be highly variable in its phylogeny and morphology, and the question arose whether the genus is monophyletic, given that the LSU region has limited resolution across taxa in this complex. A further impediment was the lack of ex-type cultures linked to older species names, that were subsequently assumed to be cosmopolitan in their distribution.

In the present study we re-examined the morphological characteristics of numerous *Pseudocercospora* species (including types) collected from Japan and Taiwan. In addition, we constructed a phylogenetic tree based on multi-locus sequences composed of ITS, *actA* and *tef1* genes to delineate species, as well as the partial DNA-directed RNA polymerase II second largest subunit gene (*rpb2*), a locus that proved to be more robust than the LSU gene in delineating genera in the Mycosphaerellaceae (Videira et al. 2015).

Materials and methods

Collection

Symptomatic leaves with leaf blight and/or spots associated with *Pseudocercospora* caespituli were collected. Samples were pressed and dried for 3–5 days between newspaper sheets, which were changed daily. Finally, the leaves were transferred to herbarium packets. Japanese specimens were deposited in the herbarium of the Graduate school of Bioresources, Mie University (TSU), Tsu, Japan. Taiwanese specimens were deposited at the herbarium of the National Chung Hsing University, Taichung, Taiwan. Single conidium isolates were cultivated on malt extract agar (MEA; Difco). Using a flame-sterilized micro-spatula, conidia were collected from caespituli or from affected leaves and suspended in sterilized water on a microscope slide. The conidial suspension was further diluted in sterilized water and pipetted onto 2 % aqueous agar in a Petri dish, and spread over the surface. After incubation at 20 °C in the dark for 24 h, the germinating conidia were individually transferred onto MEA plates using a flame-sterilized micro-hollow-cylinder under a light microscope. Purified cultures are maintained in Genebank, National Institute of Agrobiological Sciences (MAFF), Tsukuba, Japan. Some type specimens and their isolates were borrowed from the Forestry and Forest Products Research Institute or MAFF Genebank, Tsukuba, Japan.

DNA sequencing and phylogenetic analyses

Genomic DNA was extracted from cultures (Table 1) grown for 2 weeks on MEA at 25 °C. DNA was extracted using an UltraClean Microbial DNA isolation kit (MoBio Laboratories, Inc., CA, USA), according to the manufacturer's instructions. The following genes were partially amplified and sequenced: ITS, *rpb2*, *actA*, and *tef1* using the primer sets listed in Table 2. The PCR amplifications were performed using a BioRad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., CA, US). The ITS and *rpb2* PCR mixtures consisted of 1–10 ng genomic DNA, 1.25 μL 10× NH_4 Reaction Buffer (Bioline), 2.5 mM $MgCl_2$, 10 μM each dNTP, 0.16 μM each primer and 0.25 units Bioline *Taq* DNA Polymerase (Bioline

Table 1 Sources of fungal material and sequence database accession numbers

		Morphological observation	Isolates accession numbers [1]	Source of isolate	Country	Host	Family	GenBank Accession Numbers[2]			
								actA	ITS	*tef1*	*rpb2*
Cercospora	*cf nicotianae*	(not seen)	CPC 15918, CBS 132632		Mexico	*Glycine max*	Fabaceae	JX143144	JX143631	JX143390	**KX462612**
Cercospora	*oroxyli*	(not seen)	CPC 17310		Laos	*Oroxylum indicum*	Bignoniaceae	**KX462549**	**KX462582**	**KX462668**	**KX462614**
Pallidocercospora	*heimioides*	(not seen)	CBS 111190	ex-holotype	Indonesia	*Eucalyptus* sp.	Myrtaceae	DQ147633	AF309609	DQ211669	**KX462615**
Pseudocercospora	*amelanchieris*	holotype*	MUCC885, MAFF237782	ex-holotype	Japan	*Amelanchier canadenssis*	Rosaceae	**KX462550**	**KX462583**	**KX462669**	**KX462616**
Pseudocercospora	*araliae*	epitype	MUCC873	ex-epitype	Japan	*Aralia elata*	Araliaceae	GU320361	GU269653	GU384371	**KX462617**
Pseudocercospora	*cercidicola*	holotype	MUCC896, MAFF237791	ex-holotype	Japan	*Cercis chinensis*	Fabaceae	GU320377	GU269671	GU384388	**KX462618**
Pseudocercospora	*chibaensis*	holotype & epitype*	MUCC1670	ex-epitype	Japan	*Nyssa sylvatica*	Nyssaceae	**KX462551**	**KX462584**	**KX462670**	**KX462619**
Pseudocercospora	*chionanthi-retusi*	epitype*	TUA50, NCHUPP L1605	ex-epitype	Taiwan	*Chionanthus retusus*	Oleaceae	**KX462552**	**KX462585**	**KX462671**	**KX462620**
Pseudocercospora	*corylopsidis*	Isotype & epitype	MUCC908, MAFF237795	ex-epitype	Japan	*Corylopsis spicata*	Hamamelidaceae	GU320390	GU269684	GU384401	**KX462621**
Pseudocercospora	*cotoneastri*	holotype	MUCC1416, MAFF410089	ex-holotype	Japan	*Cotoneaster salicifolius*	Rosaceae	**KX462553**	**KX462586**	**KX462672**	**KX462622**
Pseudocercospora	*crispans*	(not seen)	CPC 14883, CBS 125999	ex-holotype	SouthAfrica	*Eucalyptus* sp.	Myrtaceae	GU320510	GU269807	GU384518	**KX462623**
Pseudocercospora	*cyathicola*	(not seen)	CPC 17047, CBS 129520	ex-holotype	Australia	*Cyathea australis*	Cyatheaceae	**KX462554**	JF951139	**KX462673**	**KX462624**
Pseudocercospora	*daphniphylli*	holotype	MUCC1399, MAFF410009	ex-holotype	Japan	*Daphniphyllum macropodum*	Daphniphyllaceae	**KX462555**	**KX462587**	**KX462674**	**KX462625**
Pseudocercospora	*davidiicola*	holotype	MUCC296, MAFF 240281	ex-holotype	Japan	*Davidia involucrata*	Nyssaceae	GU320398	GU269693	GU384409	**KX462626**
Pseudocercospora	*elaeocarpicola*	holotype	MUCC1236, MAFF237189	ex-holotype	Japan	*Elaeocarpus japonicus*	Elaeocarpaceae	**KX462556**	**KX462588**	**KX462675**	**KX462627**
Pseudocercospora	*eriobotryae*	lectotype* & epitype*	MUCC1007	ex-epitype	Japan	*Eriobotrya japonica*	Rosaceae	**KX462557**	**KX462589**	**KX462676**	**KX462628**
Pseudocercospora	*eriobotryicola*	epitype*	TUA12, NCHUPP L1601	ex-epitype	Taiwan	*Eriobotrya japonica*	Rosaceae	**KX462558**	**KX462590**	**KX462677**	**KX462629**
Pseudocercospora	*eupatorii-formosani*	epitype*	TUA59, NCHUPP L1606	ex-epitype	Taiwan	*Eupatorium sp.*	Aseteraceae	**KX462559**	**KX462591**	**KX462678**	**KX462630**
Pseudocercospora	*fijiensis*	(not seen)	CPC16301		Mexico	*Musa* sp.	Musaceae	**KX462548**	**KX462581**	**KX462667**	**KX462613**
Pseudocercospora	*fraxinites*	general	TUA71, NCHUPP L1607		Taiwan	*Fraxinus formosana*	Oleaceae	**KX462560**	**KX462592**	**KX462679**	**KX462631**
Pseudocercospora	*fukuokaensis*	holotype & epitype	MUCC887, MAFF237768	ex-epitype	Japan	*Styrax japonicus*	Styracaceae	GU320418	GU269714	GU384430	**KX462632**
Pseudocercospora	*hachijokibushii*	holotype	MUCC1337, MAFF238479	ex-holotype	Japan	*Stachyurus praecox* var. *matsuzakii*	Stachyuraceae	**KX462561**	**KX462593**	**KX462680**	**KX462633**
Pseudocercospora	*haiweiensis*	(not seen)	CBS 131584	ex-holotype	China	*Eucalyptus* sp.	Myrtaceae	GU320506	GU269803	GU384514	**KX462634**
Pseudocercospora	*hiratsukana*	epitype	MUCC1105, MAFF238300	ex-epitype	Japan	*Dioscorea tokoro*	Dioscoreaceae	**KX462562**	**KX462594**	**KX462681**	**KX462635**
Pseudocercospora	*houttuyniae*	holotype & epitype*	MUCC1289, MAFF238071	ex-epitype	Japan	*Houttuynia cordata*	Saururaceae	**KX462563**	**KX462595**	**KX462682**	**KX462636**
Pseudocercospora	*humuli*	holotype & epitype	MUCC742	ex-epitype	Japan	*Humulus lupulus* var. *lupulus*	Cannabaceae	GU320428	GU269725	GU384439	**KX462637**
Pseudocercospora	*imazekii*	holotype & epitype*	MUCC1668	ex-epitype	Japan	*Kolkwitzia amabilis*	Caprifoliaceae	**KX462564**	**KX462596**	**KX462683**	**KX462638**
Pseudocercospora	*iwakiensis*	holotype*	MUCC1736	ex-holotype	Japan	*Ilex crenata*	Aquifoliaceae	**KX462574**	**KX462607**	**KX462693**	**KX462657**
Pseudocercospora	*izuohshimense*	holotype	MUCC1336, MAFF238478	ex-holotype	Japan	*Helwingia japonica*	Helwingiaceae	**KX462565**	**KX462597**	**KX462684**	**KX462639**
Pseudocercospora	*kadsurae*	holotype & epitype*	MUCC752	ex-epitype	Japan	*Kadsura japonica*	Schisandraceae	**KX462566**	**KX462598**	**KX462685**	**KX462640**
Pseudocercospora	*lonicericola*	epitype* (Former neotype)	MUCC889, MAFF237785	ex-epitype	Japan	*Lonicera gracilipes var. glabra*	Caprifoliaceae	GU320438	GU269736	JQ324999	**KX462641**
Pseudocercospora	*lyoniae*	holotype & epitype*	MUCC910, MAFF237775	ex-epitype	Japan	*Lyonia ovalifolia* var. *elliptica*	Ericaceae	GU320441	GU269739	GU384451	**KX462642**
Pseudocercospora	*madagascariensis*	(not seen)	CBS 124155	ex-holotype	Madagascar	*Eucalyptus camaldulensis*	Myrtaceae	KF253625	GQ852767	KF253265	**KX462643**
pseudocercospora	*naitoi*	holotype & epitype*	MUCC1072, MAFF237906	ex-epitype	Japan	*Ilex serrata* f. *argutidens*	Aquifoliaceae	**KX462567**	**KX462599**	**KX462686**	**KX462644**
Pseudocercospora	*nandinae*	epitype*	MUCC1260, MAFF239633	ex-epitype	Japan	*Nandina domestica*	Berberidaceae	**KX462568**	**KX462600**	**KX462687**	**KX462645**
Pseudocercospora	*nephrolepidicola*	(not seen)	CPC 17049, CBS 128211	ex-holotype	Australia	*Nephrolepis falcata*	Davalliaceae	**KX462569**	HQ599590	**KX462688**	**KX462646**
Pseudocercospora	*neriicola*	(not seen)	CPC 23765, CBS 138010	ex-holotype	Italy	*Nerium oleander*	Apocynaceae	KJ869231	KJ869165	KJ869240	**KX462647**
Pseudocercospora	*norchiensis*	(not seen)	CBS 120738	ex-holotype	Italy	*Rubus* sp.	Rosaceae	GU320455	EF394859	GU384464	**KX462648**
Pseudocercospora	*paederiae*	general	MUCC1355, MAFF239161		Japan	*Paederia foetida*	Rubiaceae	**KX462570**	**KX462603**	**KX462689**	**KX462651**
Pseudocercospora	*palleobrunnea*	(not seen)	CPC 13387, CBS 124771	ex-holotype	Australia	*Syzygium* sp.	Myrtaceae	GU320500	GQ303288	GU384509	**KX462652**
Pseudocercospora	*photiniae*	neotype*	MUCC1661	ex-neotype	Japan	*Photinia glabra*	Rosaceae	**KX462571**	**KX462604**	**KX462690**	**KX462653**
Pseudocercospora	*pouzolziae*	general	TUA80, NCHUPP L1608		Taiwan	*Pouzolzia* sp.	Urticaceae	**KX462572**	**KX462605**	**KX462691**	**KX462654**
Pseudocercospora	*punicae*	general	MUCC1209, MAFF236998		Japan	*Punica granatum*	Lythraceae	**KX462573**	**KX462606**	**KX462692**	**KX462655**
Pseudocercospora	*robusta*	(not seen)	CBS 111175	ex-holotype	Malaysia	*Eucalyptus robur*	Myrtaceae	DQ147617	AY309597	DQ211683	**KX462656**
Pseudocercospora	sp.	(not seen)	TUA29		Taiwan	*Sterculia ceramica*	Malvaceae	**KX462576**	**KX462602**	**KX462695**	**KX462650**
Pseudocercospora	sp.	general	TUA31, NCHUPP L1602		Taiwan	*Pyracantha coccinea*	Rosaceae	**KX462575**	**KX462601**	**KX462694**	**KX462649**
Pseudocercospora	*stephanandrae*	holotype & epitype*	MUCC914, MAFF237799	ex-epitype	Japan	*Stephanandra incisa*	Rosaceae	GU320516	GU269814	GU384526	**KX462658**

Table 1 (continued)

		Morphological observation	Isolates accession numbers [1]	Source of isolate	Country	Host	Family	GenBank Accession Numbers [2]			
								actA	ITS	*tef1*	*rpb2*
Pseudocercospora	*tereticornis*	(not seen)	CPC 13299, CBS 125214	ex-holotype	Australia	*Eucalyptus tereticornis*	Myrtaceae	GU320499	GQ852770	GU384508	**KX462659**
Pseudocercospora	*tinea*	epitype*	TUA40, NCHUPP L1603	ex-epitype	Taiwan	*Viburnum* sp.	Adoxaceae	**KX462577**	**KX462608**	**KX462696**	**KX462660**
Pseudocercospora	*tinea*	general	TUA56, NCHUPP L1604		Taiwan	*Viburnum* sp.	Adoxaceae	**KX462578**	**KX462609**	**KX462697**	**KX462661**
Pseudocercospora	*violamaculans*	neotype*	MUCC1660	ex-neotype	Japan	*Rhaphiolepis indica*	Rosaceae	**KX462579**	**KX462610**	**KX462698**	**KX462662**
Pseudocercospora	*vitis*	general	CPC 11595, CBS 132012		South Korea	*Vitis vinifera*	Vitaceae	GU320533	GU269829	GU214483	**KX462663**
Pseudocercospora	*xenosyzygiicola*	lectotype* & epitype*	MUCC1481, MAFF237986	ex-epitype	Japan	*Syzygium samarangense*	Myrtaceae	**KX462580**	**KX462611**	**KX462699**	**KX462664**
Pseudocercospora	*zelkovae*	general	MUCC872, MAFF238237		Japan	*Zelkova serrata*	Ulmaceae	GU320537	GU269835	GU384547	**KX462665**
Trochophora	*simplex*	general	CBS 124744		South Korea	*Daphniphyllum macropodum*	Daphniphyllaceae	GU320568	GU269872	GU384580	**KX462666**

*newly designated types in this study

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan. TUA: acting cultures in Lab. of Electron Mycroscopy, Tokyo University of Agriculture, Tokyo, Japan; NCHUPP: the herbarium, Department of National Chung Hsing University, Taichung, Taiwan

actA: partial actin gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrRNA gene; *tef1*: partial translation elongation factor 1-alpha gene; *rpb2*: partial RNA polymerase II second largest subunit gene

[2] newly obtained sequeces are indicated in bold

GmbH Luckenwalde, Germany) in a total volume of 12.5 μL. The *actA* PCR mixture consisted of 1–10 ng genomic DNA, 1.25 μL 10× NH_4 Reaction Buffer (Bioline), 2 mM $MgCl_2$, 5 μM each dNTP, 0.2 μM each primer and 0.25 units Bioline *Taq* DNA Polymerase (Bioline) in a total volume of 12.5 μL. The *tef1* PCR mixtures consisted of 10–20 ng genomic DNA, 1.25 μL 10× NH_4 Reaction Buffer (Bioline), 0.7 μL Dimethyl sulfoxide (DMSO) (99.9 %), 2 mM $MgCl_2$, 20 μM each dNTP, 0.2 μM each primer and 0.5 units Bioline *Taq* DNA Polymerase (Bioline) in a total volume of 12.5 μL. The PCR conditions consisted of an initial denaturation (94 °C, 3 min), and 40 cycles of amplification (94 °C, 30 s; annealing (Table 2), 30 s; 72 °C, 45 s), and final extension (72 °C, 5 min). To obtain partial *rpb2* amplicons, a touchdown PCR protocol was used, with an initial denaturation (94 °C, 3 min), and then five amplification cycles (94 °C, 45 s; 60 °C, 45 s; 72 °C, 2 min), five amplification cycles (94 °C, 45 s; 58 °C, 45 s; 72 °C, 2 min), 30 amplification cycles (94 °C, 45 s; 54 °C, 45 s; 72 °C, 2 min), and a final extension (72 °C, 8 min). The resulting fragments were sequenced in both directions using the PCR primers and a BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies). DNA sequencing amplicons were purified with Sephadex G-50 Superfine columns (Sigma-Aldrich) in MultiScreen HV plates (Millipore). Purified products were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies). The DNA sequences were analysed, and consensus sequences were computed and concatenated using MEGA v. 5.2 software (Tamura et al. 2011).

Trochophora simplex (CBS 124744) and *Pallidocercospora heimioides* (ex-type: CBS 111190) were selected as outgroups for the *Pseudocercospora* alignment. The sequences for each gene were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html). The alignments were manually checked and improved where necessary using MEGA v. 5.2, after which the sequences were concatenated. Parsimony and Bayesian analyses were used to estimate phylogenetic relationships in the combined dataset. Parsimony analyses were conducted with PAUP v. 4.0b10 (Swofford 2003). Alignment gaps were treated as fifth bases and all characters were unordered and of equal weight. The robustness of the obtained trees was evaluated by 1 000 bootstrap replications (Hillis and Bull 1993). Kakusan4 (Tanabe 2011) was used to determine the best nucleotide substitution model settings for each data partition in order to perform a model-optimised Bayesian phylogenetic reconstruction using MrBayes v. 3.2.5 (Ronquist et al. 2012). The heating chain was set at 0.1, and Markov Chain Monte Carlo (MCMC) analyses of four chains were performed in parallel from a random tree topology, terminating

Table 2 Details of primers used in this study for amplification and sequencing

Locus	Primer	Sequence (5′–3′)	Orientation	Annealing Temperature (°C)	Reference
ITS	V9G	TTA CGT CCC TGC CCT TTG TA	Forward	48	de Hoog and Gerrits van den Ende (1998)
	ITS4	TCC TCC GCT TAT TGA TAT GC	Reverse	48	White et al. (1990)
rpb2	RPB2-5f2	GGGGWGAYCAGAAGAAGGC	Forward	54–60	Sung et al. (2007)
	fRPB2-5 F	GAYGAYMGWGATCAYTTYGG	Forward	54–60	Liu et al. (1999)
	fRPB2-7cR	CCCATRGCTTGTYYRCCCAT	Reverse	54–60	Liu et al. (1999)
actA	ACT-512 F	ATG TGC AAG GCC GGT TTC GC	Forward	48	Carbone and Kohn (1999)
	ACT-783R	TAC GAG TCC TTC TGG CCC AT	Reverse	48	Carbone and Kohn (1999)
tef1	EF1-728 F	CAT CGA GAA GTT CGA GAA GG	Forward	52	Carbone and Kohn (1999)
	EF1-986R	TAC TTG AAG GAA CCC TTA CC	Reverse	52	Carbone and Kohn (1999)

when the average standard deviation of split frequencies reached a value of 0.01.

Results

Phylogeny

A concatenated alignment consisting of 50 strains, including sequences obtained from GenBank, were analyzed. The alignment contained a total of 1,842 characters, including gaps, which were treated as "fifth bases". For the parsimony analyses, 927 characters were constant and 704 characters were parsimony-informative. One of the 47 equally most parsimonious trees (TL = 3,434, CI = 0.4462, RI = 0.7027, RC = 0.3135) was selected based on the result of the Kishino-Hasegawa (KH) test (Kishino and Hasegawa 1989) in PAUP and shown in Fig. 1. In the Bayesian analyses, the optimum evolutionary model for each locus, ITS, *actA*, *tef1*, and *rpb2*, was selected by KAKUSAN4 (Tanabe 2011). These optimal models were the K80-Gamma model (Kimura 1980) for ITS and *rpb2*, SYM-Gamma (Zharkikh 1994) for *actA*, and HKY85 (Hasegawa et al. 1985) for *tef1*. The Bayesian analysis was conducted for 6,000,000 generations. Trees were saved every 500 generations, resulting in 12,001 trees saved. Of these, 5,582 trees were considered "burn-in," after which the likelihood values were stationary. The Bayesian 50 % majority rule consensus tree of these posterior-sampled trees was generated in MrBayes v. 3.2.5 (tree not shown). The Bayesian posterior probabilities were calculated and indicated on the nodes of the most parsimonious tree generated in this study (Fig. 1). Trees from both analyses were visualized in FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree). The resulting tree and respective alignment were deposited in TreeBASE at www.treebase.org (http://purl.org/phylo/treebase/phylows/study/TB2:S19478). The tree topologies of trees generated in both analyses were similar, and the major clades were supported by both bootstrap and posterior probability values (Fig. 1).

Taxonomy

Pseudocercospora amelanchieris C. Nakash., Tak. Kobay. & Crous, **sp. nov**. [**MB 817410**] Fig. 2a, b, c, 4 k

Etymology: Named after the host genus from which it was isolated, *Amelanchier*.

Description: *Leaf spots* pale brown to brown with yellow halo, circular to irregular, 2–20 mm diam. *Caespituli* amphigenous, mainly hypophyllous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 1.5–2 μm wide, septate, hyaline to pale olivaceous brown, thin-walled, smooth. *Stromata* lacking to moderate, amphigenous, mainly hypophyllous, epidermal, erumpent, stomatal, pale brown to brown, to 48 μm diam, often with external hyphae on the lower leaf surface. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, loosely fasciculate from stomata, or solitary from external hyphae, pale olivaceous to pale brown, simple or branched, straight to geniculate due to sympodial sporulation, smooth, thin-walled, 0–3-septate, 1.5–38 × 1.8–2 μm. *Conidiogenous cells* integrated, terminal or intercalary, proliferating sympodially, sporulating polyblastically, with unthickened loci, 1–1.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, cylindrical to long obclavate, smooth to rough, straight to curved, thin-walled, obconically truncate at the base, acutely rounded to rounded at the apex, 1–5-euseptate, 20–67 × 2–4 μm, with unthickened and not darkened hilum, 1–1.5 μm diam.

Hosts: On leaves of *Amelanchier canadensis* (L.) Medik., *Amelanchier asiatica* (Siebold & Zucc.) Endl. ex Walp. (Rosaceae).

Material examined: **Japan**, Ibaraki, Tsukuba, on *Amelanchier canadensis*, 11 Sept. 1998, T. Kobayashi, C. Nakashima, E. Imaizumi, & K. Motohashi (holotype TSU-MUMH11539; ex-holotype culture MUCC885 = MAFF237782).

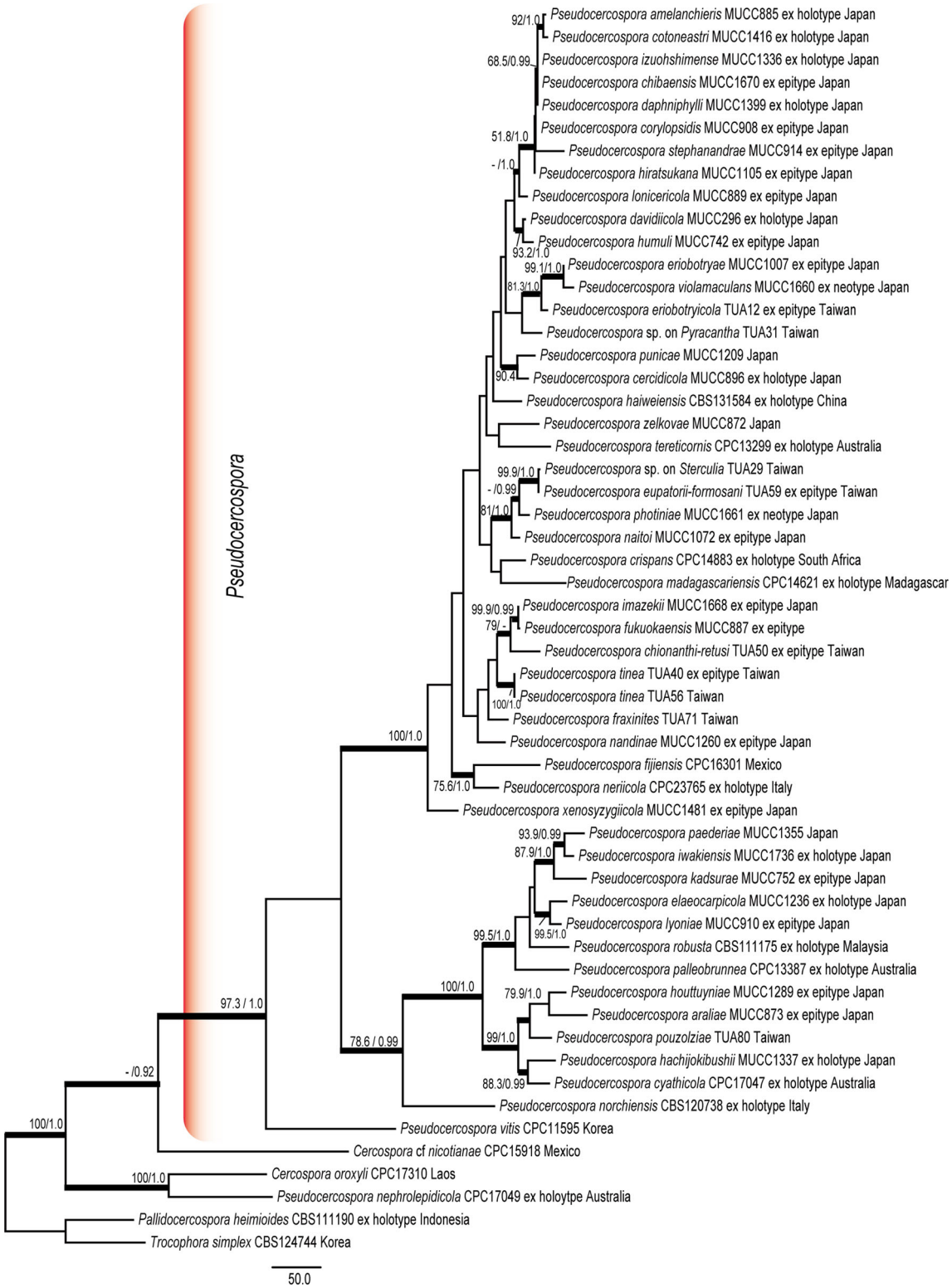

Fig. 1 Phylogenetic tree showing 1 of 47 equally most parsimonious MP trees, 3,434 steps long, generated by PAUP from the analysis of the concatenated alignment composed of ITS, *actA*, *tef1*, and *rpb2* sequences of *Pseudocercospora* species. MP bootstrap support values above 60 % and Bayesian posterior probabilities are given above or below the nodes

Notes: *Pseudocercospora amelanchieris* is the first *Pseudocercospora* species on *Amelanchier*. Another specimen of a *Pseudocercospora* sp. on *Amelanchier asiatica*, which is characterized mainly by epiphyllous and well-developed stromata lacking external hyphae, was collected by C. Nakashima (TSU-MUCNS128) in Japan. The

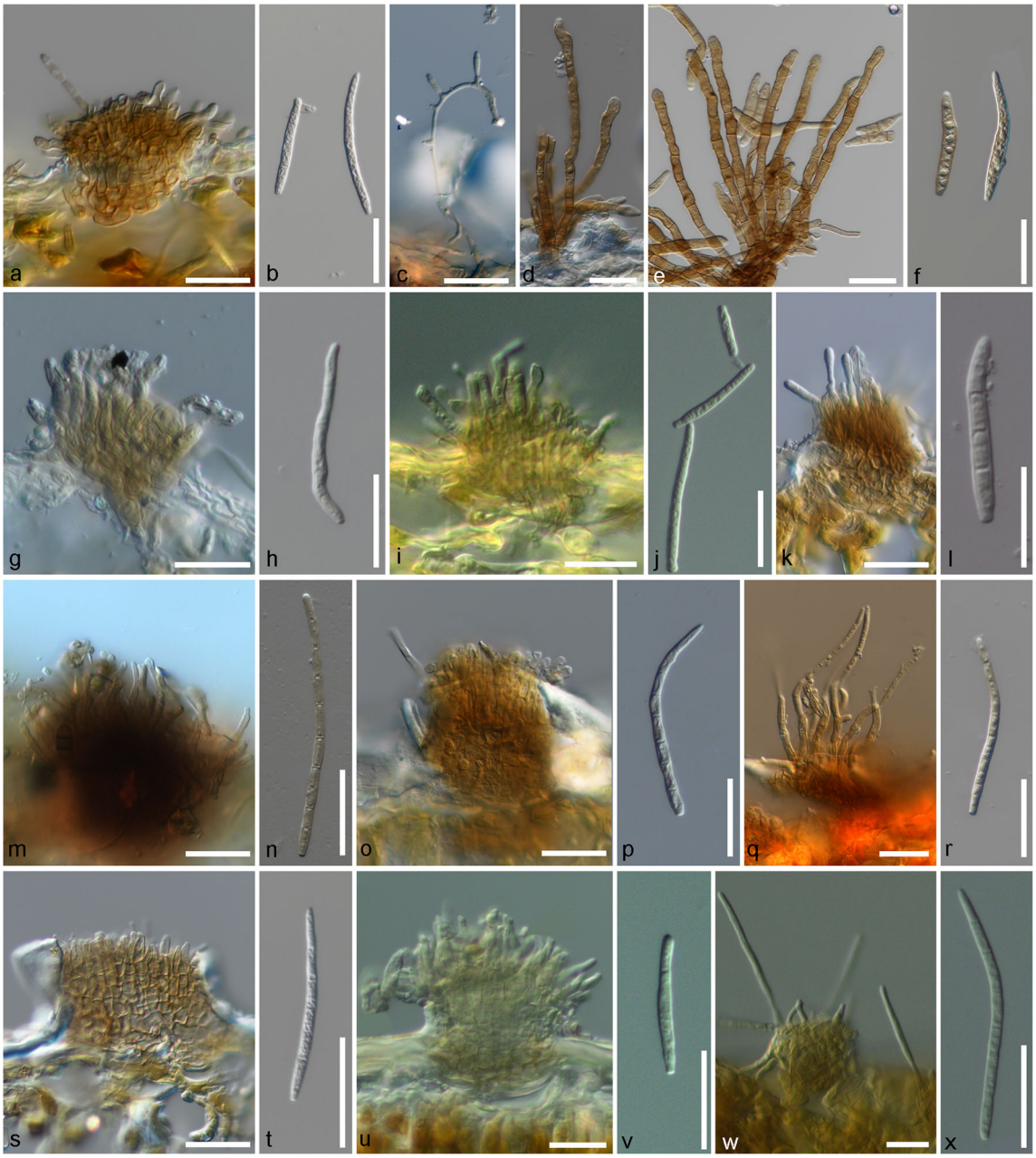

Fig. 2 *Pseudocercospora amelanchieris*. **a** Stroma and conidiophores. **b** Conidia. **c** External hyphae with conidiophores. *Ps. araliae*. **d** Stroma and conidiophores. **e** Conidiophores. **f** Conidia. *Ps. chibaensis*. **g** Stroma and conidiophores. **h** Conidium. *Ps. chionanthi-retusi*. **i** Stroma and conidiophores. **j** Conidia. *Ps. corylopsidis*. **k** Stroma and conidiophores. **l** Conidium. *Ps. cotoneastri*. **m** Stroma and conidiophores. **n** Conidium. *Ps. daphniphylli*. **o** Stroma and conidiophores. **p** Conidium. *Ps. elaeocarpicola*. **q** Stroma and conidiophores. **r** Conidium. *Ps. eriobotryae*. **s** Stroma and conidiophores. **t** Conidium. *Ps. eriobotryicola*. **u** Stroma and conidiophores. **v** Conidium. *Ps. eupatorii-formosani*. **w** Stroma and conidiophores. **x** Conidium. *Scale bars* 20 μm

phylogenetic relationship of this specimen was not determined in the present study, and further collections are required to elucidate all *Pseudocercospora* spp. on *Amelanchier*. In the combined tree (Fig. 1, ESM 1) *Ps. amelanchieris* locates as sister to *Ps. cotoneastri* on *Cotoneaster salicifolius* (Rosaceae), which has somewhat larger and narrower conidia. Although morphological characteristics of caespituli barely distinguish *Ps. amelanchieris* from *Ps. cotoneastri*, those hosts and phylogenetic relationships support that these fungi should be treated as two different species.

Pseudocercospora araliae (Henn.) Deighton, Mycol. Pap. 140: 19, 1976. Figure 2d, e, f

≡ *Cercospora araliae* Henn., Beibl. Bot. Jahrb. Syst. 31: 742, 1902.

≡ *Cercosporiopsis araliae* (Henn.) Miura, Fl. Manchuria & E. Mongolia 27, fungi 3: 533, 1928.

Description: *Leaf spot* indistinctly zonate, yellow to dark brown, 5–10 mm diam, later confluent and larger. *Caespituli* hypophyllous, sooty or velutinous by well-formed conidiophores and conidia. *Mycelium* internal and external; external hyphae emerging through stomata or arising from small stromata, branched, 2–5 μm wide, septate, hyaline to brown, thick-walled, smooth to rough. *Stromata* lacking to small, composed of few brown cells, epidermal, stomatal. *Conidiophores* loosely fasciculate (1–16), emerging through stomata, brown to dark brown, simple or branched, straight to geniculate caused by sympodial sporulation, smooth to rough, thick-walled, 0–17-euseptate, 19–200 × 4.5–7 μm. *Conidiogenous cells* integrated, terminal and intercalary, proliferating sympodially or percurrently, sporulating polyblastically with distinct, not refractive, not darkened or somewhat darkened, unthickened and truncate loci at the shoulders, 2–2.5 μm diam. *Conidia* solitary, holoblastic, subhyaline to brown, cylindrical to obclavate, smooth to rough, frequently constricted at septa, straight to curved, thick-walled, obconically truncate, often protruding at the base, rounded or acutely rounded at the apex, often beak-like, 1–10-euseptate, 26–72 × 4–7 μm, with unthickened and not darkened hilum, 2–2.5 μm diam.

Host: On leaves of *Aralia elata* (Miq.) Seem. (Araliaceae).

Type: Japan, Tosa (Kochi Prefecture), Ushioe-yama, on leaves of *Aralia elata* var. glabrescens, Aug. 1901, T. Yoshinaga (holotype B 700015014).

Material examined: **Japan**, Toyama Prefecture, Asahi, on leaves of *Aralia elata*, 1 Oct. 1996, T. Kobayashi & C. Nakashima (epitype designated in Crous et al. (2013), TFM:FPH-8094 = TSU-MUMH11382; ex-epitype culture MUCC873 = MAFF238192).

Pseudocercospora cercidicola Crous, U. Braun & C. Nakash., Stud. Mycol. 75: 79, 2013.

Description and illustration: See Crous et al. (2013).

Host: On leaves of *Cercis chinensis* Bunge (Fabaceae).

Material examined: **Japan**, Ibaraki, on leaves of *Cercis chinensis*, 10 Sept. 1998, T. & K. Kobayashi (holotype CBS-H20895; ex-holotype culture MUCC896 = MAFF237791).

Notes: In Japan, two *Pseudocercospora* species, *Ps. cercidicola* and *Ps. cercidis-chinensis*, are found on *Cercis*. These species are distinguished based on the phylogenetic relationship and the size of conidia and external hyphae in vivo. Crous et al. (2013), noted that *Cercospora cercidis* Y. Nisik. (heterotypic synonym of *Ps. cercidis-chinensis*) was recognized as a Japanese species on *Cercis*. However, the origin of *Cercospora cercidis* was Nanjing (Nanking), China.

Pseudocercospora chibaensis Tak. Kobay. & Nagash., Trans. Mycol. Soc. Japan 32: 328, 1991. Figures 2g, h, and 4l

Description: *Leaf spots* pale brown to greyish brown with reddish brown to purple border, circular to irregular, 2–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata on the lower leaf surface, branched, 2–3 μm wide, septate, hyaline to pale brown, thin-walled, smooth. *Stromata* small to well-developed, amphigenous, mainly epiphyllous, epidermal, erumpent, stomatal, pale brown to brown, to 65 μm diam. *Conidiophores* densely fasciculate, emerging from stromata, or solitary from external hyphae, subhyaline to pale brown, simple, straight to geniculate, smooth, somewhat thick-walled, irregular in width, 0–2-septate, 8–33 × 2–4.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with unthickened loci, 1.5–3 μm diam. *Conidia* solitary, holoblastic, pale brown, variable in shape, obovoid, cylindrical, acicular to obclavate, somewhat thick-walled, short to long obconically truncate at the base, rounded at the apex, 0–8-euseptate, 15–55 × 3.5–5 μm, with unthickened and not darkened hilum, 1.5–3 μm diam.

Host: On leaves of *Nyssa sinensis* Oliv., *N. sylvatica* Marshall (Nyssaceae).

Type (examined): Japan, Chiba Prefecture, Matsudo, on leaves of *Nyssa sinensis*, 7 Nov. 1987, M. Nagashima & T. Kobayashi (holotype TFM:FPH-6914).

Material examined: **Japan**, Kanagawa Prefecture, Kamakura, on leaves of *N. sylvatica*, 1 Nov. 2012, M. Abe, Y. Koba, & H. Horie (epitype **designated here** TSU-MUMH11420, MycoBank MBT372137; ex-epitype culture MUCC1670).

Note: Caespituli in the epitype were somewhat darker in colour than those on the holotype. *Ps. chibaensis* was described from Japan on *Nyssa sinensis*, a deciduous tree native in China and Vietnam. Braun et al. (2016b) recently recorded this cercosporoid species from China.

Pseudocercospora chionanthi-retusi Goh & W.H. Hsieh, *Cercospora* and similar fungi from Taiwan: 249, 1990. Figures 2i, j, and 4m

= *Cercospora chionanthi-retusi* Togashi & Katsuki, Sci. Rept. Yokohama Nat. Univ. Sec.II. 1: 1, 1952.

≡ *Pseudocercospora chionanthi-retusi* (Togashi & Katsuki) Nishijima, C. Nakash. & Tak. Kobay., Mycoscience 40: 270, 1999 (non *Ps. chionanthi-retusi* Goh & Hsieh in Hsieh & Goh, 1990).

≡ *Pseudocercospora chionanthicola* C. Nakash. & Tak. Kobay., Mycoscience 43: 98, 2002.

Description: *Leaf spots* pale brown to greyish brown, angular to irregular, vein limited, scattered, 1–6 mm. *Caespituli* hypophyllous. *Mycelium* internal, subhyaline to pale brown. *Stromata* lacking to moderate, hypophyllous, composed of a few pale brown to brown cells, or moderately developed, epidermal, erumpent, stomatal, pale olivaceous brown, to 40 μm

diam. *Conidiophores* solitary from substomatal hyphae, or loosely to densely fasciculate arising from stromata, simple or branched, straight or geniculate caused by sympodial proliferation, smooth to rough, thin-walled, cylindrical or irregular in width, 0–2-septate, 10–40 × 2–4.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with truncate and unthickened loci at the apex and shoulders caused by sympodial proliferation, 1.5–2.5 μm diam. *Conidia* solitary, holoblastic, hyaline to very pale olivaceous, cylindrical to obclavate, smooth, straight to slightly curved, thin-walled, short- to long-obconically truncate at the base, rounded at the apex, 2–7-septate, 16–44 × 2–4 μm, with unthickened and not darkened hilum, 1.5–2.5 μm diam.

Host: On leaves of *Chionanthus retusus* Lindl. & Paxton (Oleaceae).

Type: Taiwan, Taipei, on leaves of *Chionanthus retusus*, 29 Aug. 1986, T.K. Goh (NCHUPP-23; missing in the National Chung Hsing University), illustration in Goh & Hsieh (1990, fig. 191, **designated here** as lectotype, MBT372396).

Material examined: **Taiwan**, Taichung, National Science Museum, on leaves of *Chionanthus retusus*, 8 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (epitype **designated here** TUA50 = NCHUPP 3205, MycoBank MBT372138; culture NCHUPP L1605).

Notes: In this study, we could not re-examine the type material collected from Taipei (NCHUPP-23), as the specimen is missing. However, we designate the original illustration as lectotype, to facilitate epitypification. The morphological characteristics of the samples collected in Taichung were identical to those of the protolog of *Pseudocercospora chionanthiretusi*, and therefore this specimen represents a suitable epitype.

Pseudocercospora corylopsidis (Togashi & Katsuki) C. Nakash. & Tak. Kobay., Mycoscience 40: 270, 1999. Figure 2k, l

≡ *Cercospora corylopsidis* Togashi & Katsuki, Bot. Mag. (Tokyo) 65: 20, 1952.

Description: *Leaf spots* brown, angular to irregular, later enlarged and confluent, 3–10 mm. *Caespituli* amphigenous. *Mycelium* internal or external; external hyphae emerging through stomata or arising from stromata, branched, 2–3 μm wide, septate, subhyaline to pale olivaceous brown, thin-walled, smooth to rough. *Stromata* small to moderate, mainly epiphyllous, epidermal, erumpent, stomatal, pale brown to brown, 28–50 μm diam, often with external hyphae. *Conidiophores* densely fasciculate, emerging from stromata, or solitary from external hyphae, subhyaline to pale brown, simple or branched, straight to geniculate, smooth to rough, somewhat thick-walled at the base, cylindrical, 0–2-septate, 6–23 × 2–4 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with unthickened and indistinct loci, 1.5–3 μm diam. *Conidia* solitary, hyaline to subhyaline, cylindrical to obclavate, straight to slightly curved, smooth to rough, thin-walled, obconically truncate at the base, rounded at the apex, 2–5-euseptate, 17–63 × 3–4 μm, with unthickened and not darkened hilum, 1.5–3 μm diam.

Hosts: On leaves of *Corylopsis pauciflora* Siebold & Zucc., *C. spicata* Siebold & Zucc. (Hamamelidaceae).

Type: Japan, Kagoshima Prefecture, Kagoshima, on leaves of *Corylopsis pauciflora*, 26 Oct. 1949, S. Katsuki (holotype YNU; isotype TNS-F-243824).

Materials examined: **Japan**, Kagoshima Prefecture, Kagoshima, on leaves of *Corylopsis pauciflora*, 26 Oct. 1949, S. Katsuki (isotype TNS-F-243824); **Japan**, Tokyo, Chofu, Jindai Bot. Garden, on leaves of *C. spicata*, 7 Nov. 1998, C. Nakashima & E. Imaizumi (epitype designated in Crous et al. (2013), TFM:FPH-8095; ex-epitype culture MUCC908 = MAFF237795).

Pseudocercospora cotoneastri (Katsuki & Tak. Kobay.) Deighton, Trans. Brit. Mycol. Soc. 88: 389, 1987. Figure 2m, n

≡ *Cercospora cotoneastri* Katsuki & Tak. Kobay., as "*cotoneasteris*", Trans. Mycol. Soc. Japan 17: 276, 1976.

Description: *Leaf spots* dark brown to black with indefinite border, circular to irregular, later enlarged, 2–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata on the lower leaf surface or arising from stromata, branched, 2–3 μm, septate, pale brown to pale olivaceous brown, smooth to rough, somewhat thick-walled. *Stromata* amphigenous, mainly epiphyllous, epidermal, erumpent, stomatal, pale brown to brown, 40–50 μm diam, often with external hyphae on the lower leaf surface. *Conidiophores* densely fasciculate arising from stromata, or solitary from well developed external hyphae creeping around the lower leaf surface, brown to pale olivaceous brown, paler towards the apex, simple or branched, straight to geniculate, smooth to rough, somewhat thick-walled, 0–2-septate, irregular in width, 3–34 × 2–3 μm. *Conidiogenous cells* integrated, terminal or intercalary, proliferating sympodially or percurrently, sporulating polyblastically, with indistinct and unthickened loci on the shoulders caused by sympodial sporulation, 1–1.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, cylindrical to long obclavate, smooth, somewhat thick-walled, obconically truncate at the base, acutely rounded to rounded at the apex, 5–9-euseptate, 40–72 × 2–3.5 μm, with unthickened and not darkened hilum, 1–1.5 μm diam,

Hosts: On leaves of *Cotoneaster dammeri* C.K. Schneid.; *C. franchetii* Bois; *C. horizontalis* Decne.; *C. salicifolius* Franch.; *C. thymifolius* Baker; *C. rotundifolius* Wall. ex Lindl.; *C. watereri* Exell; *Cotoneaster* sp. (Rosaceae).

Type (examined): Japan, Tokyo, Takao, Government Forest Experimental Station, Asakawa Experimental Forest, on leaves of *Cotoneaster salicifolius*, 13 Aug. 1974, T. Kobayashi (holotype TFM:FPH-4185; ex-holotype culture MAFF410089 = MUCC1416)

Material examined: **Japan**, Shizuoka, Hamamatsu, on leaves of *C. salicifolius*, 1 Nov. 2007, T. Kobayashi & C. Nakashima, MUCNS126.

Pseudocercospora daphniphylli (Katsuki & Tak. Kobay.) Deighton, Trans. Brit. Mycol. Soc. 88: 389, 1987. Figure 2o, p

≡ *Cercospora daphniphylli* Katsuki & Tak. Kobay., Trans. Mycol. Soc. Japan 23: 44, 1982.

Description: *Leaf spots* purplish dark brown to greyish brown with purple border on the upper leaf surface, reddish brown to purplish brown with purplish red border on the lower leaf surface, elliptical to irregular 3–10 mm. *Caespituli* amphigenous. *Mycelium* internal, branched, 2–2.5 μm wide, septate, hyaline to pale brown, thin-walled, smooth. *Stromata* well developed, amphigenous, epidermal, erumpent, substomatal, pale brown to brown, 30–80 μm diam. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, simple, straight to mildly sinuous, smooth to rough, thick-walled at the base, 0–3-septate, 13–26 × 2.5–3 μm. *Conidiogenous cells* integrated, terminal, proliferating percurrently, sporulating polyblastically, with distinct, unthickened and truncate loci, 1.5–2 μm. *Conidia* solitary, holoblastic, subhyaline to pale brown, obclavate, straight to curved, smooth, thin-walled, truncate to obconically truncate at the base, acutely rounded at the apex, 3–8-euseptate, 27–67 × 1.5–3.5 μm, with unthickened and not darkened hilum, 1.5–2 μm diam.

Host: On leaves of *Daphniphyllum macropodum* Miq. (Daphniphyllaceae).

Type (examined): Japan, Tokyo, Chofu, Jindai Bot. Garden, on leaves of *Daphniphyllum macropodum*, 25 Sept. 1974, T. Kobayashi (holotype TFM:FPH-4431; ex-holotype culture MAFF410009 = MUCC1399).

Pseudocercospora davidiicola C. Nakash., H. Horie & Tak. Kobay., Mycoscience 49: 142, 2008.

Description: *Leaf spots* pale brown to brown with concentric ring, circular to subcircular, 3–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and rarely external; external hyphae arising from stromata or emerging through stomata, branched, 2–2.5 μm wide, septate, hyaline to pale brown. *Stromata* small to well developed, mainly epiphyllous, epidermal, substomatal, pale brown to olivaceous brown, 12–57 μm diam. *Conidiophores* densely fasciculate, arising from the upper part of stromata, solitary from external hyphae, pale brown to pale olivaceous brown, simple, rarely branched, straight to sinuous, smooth, thin-walled, 0–3-septate 12–42 × 2–4 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened, inconspicuous, not darkened, not refractive loci, 1.5–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, cylindrical to obclavate, straight to slightly curved, smooth, thin-walled, truncate or obconically truncate at the base, rounded at the apex, 2–10-euseptate, 15–86 × 2.5–4 μm, with unthickened and not darkened hilum, 1.5–2 μm diam.

Host: On leaves of *Davidia involucrata* Baill. (Nyssaceae).

Type (examined): Japan, Aichi Prefecture, Higashi-yama Bot. Gard, on leaves of *Davidia involucrata*, 24 Oct. 2005, I. Araki (holotype TFM:FPH-7853; ex-type culture MUCC296 = MAFF240281).

Note: See Motohashi et al. 2008 for the note and illustrations.

Pseudocercospora elaeocarpicola Tak. Kobay., Nishij. & C. Nakash., Mycoscience 39: 187, 1998. Figure 2q, r

Description: *Leaf spots* circular to irregular, pale brown to grayish brown with reddish brown to blackish brown border, 5–10 mm. *Caespituli* epiphyllous, rarely amphigenous. *Mycelium* internal, branched, 2–2.5 μm wide, septate, pale brown. *Stromata* epiphyllous, epidermal, erumpent, brown, small to well developed, to 65 μm diam. *Conidiophores* loosely to densely fasciculate, emerging from the upper part of stromata, pale brown, paler towards apex, simple or branched, straight to sinuous-geniculate, smooth, somewhat thick-walled, 0–4-septate, irregular in width, 6–60 × 2.5–4 μm. *Conidiogenous cells* integrated, terminal or intercalary, proliferating sympodially or percurrently, sporulating polyblastically, with unthickened and truncate loci, 1.5–2.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, cylindrical to obclavate, straight to curved, smooth, somewhat thick-walled, truncate or long obconically truncate at the base, acutely rounded to rounded at the apex, 3–9-euseptate, 32–75 × 2.5–3 μm, with unthickened and nor darkened hilum, 1.5–2.5 μm diam.

Host: On leaves of *Elaeocarpus japonicus* Siebold & Zucc. (Elaeocarpaceae).

Type (examined): Japan, Okinawa Prefecture, Nago, Mt. Minami-Meiji, on leaves of *Elaeocarpus japonicus*, 10 Nov. 1994, T. Kobayashi & C. Nakashima (holotype TFM:FPH7477; ex-holotype culture MAFF237189 = MUCC1236).

Pseudocercospora eriobotryae (Enjoji) Goh & W.H. Hsieh, Trans. Mycol. Soc. Republ. China 2: 135, 1987. Figures 2s, t, and 4n

≡ *Cercosporina eriobotryae* Enjoji, J. Pl. Protect. 18: 332, 1931.

≡ *Cercospora eriobotryae* (Enjoji) Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 61: 94, 1933.

≡ *Pseudocercospora eriobotryae* (Enjoji) Y.L. Guo & X.J. Liu, Mycosystema 2: 234, 1989.

Description: *Leaf spot* brown to dark brown with pale brown border on the upper surface, circular to angular, or irregular, scattered, 2–10 mm. *Caespituli* mainly epiphyllous. *Mycelium* internal, branched, 2–2.5 μm wide, septate, hyaline to pale olivaceous brown, thin-walled, smooth. *Stromata* well developed, mainly epiphyllous, epidermal, erumpent, pale brown to brown, 34–72 μm diam. *Conidiophores* emerging from the upper part of stromata, densely fasciculate, pale brown, simple or branched, straight to mildly geniculate, smooth, thin-walled, 2–5-septate, 7–21 × 2–2.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with unthickened and truncate loci at the apex and shoulders caused by sympodial proliferation, 1–2 μm diam. *Conidia* solitary, holoblastic, hyaline to subhyaline, acicular, cylindrical to obclavate, straight to mildly curved, smooth to rough, thin- to thick-walled, long obconically truncate at the base, acutely rounded at the apex, 4–8-euseptate, 30–77 × 2–3 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Host: On leaves of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae).

Type: Japan, Chiba, Chiba Prefectural Agricultural Experiment Station, on leaves of *Eriobotrya japonica*, 1 Oct. 1930, S. Enjoji (Not preserved).

Lectotype (**designated here** MycoBank MBT372139): J. Pl. Protect. 18: 330, Figs. 1, and 2a–b, 1931 (original illustration: iconotype).

Materials examined: **Japan**, Ibaraki Prefecture, Kukisaki, Kannondai, on leaves of *Eriobotrya japonica*, 23 Oct. 1998, T. Kobayashi, MUCNS486, culture MUCC905 = MAFF237769; **Japan**, Chiba Prefecture, Ajiki, on leaves of *E. japonica*, 26 Aug. 2009, C. Nakashima & E. Nakashima (epitype **designated here** TSU-MUMH11284, MycoBank MBT372140; ex-epitype culture MUCC1007); **Japan**, Kagoshima Prefecture, Kamiyaku, on leaves of *E. japonica*, 19 Oct. 1997, T. Kobayashi & C. Nakashima, MUCNS231; **Japan**, Tokyo, Chofu, Jindai Bot. Park, on leaves of *E. japonica*, 7 Nov. 1998, C. Nakashima & E. Imaizumi, culture MAFF237769; **Japan**, Tokyo, Edogawa, on leaves of *Eriobotrya japonica*, 16 Nov. 1974, H. Horie, TFM:FPH-4300.

Pseudocercospora eriobotryicola (J.M. Yen) J.M. Yen, Bull. Soc. Mycol. France 94: 386, (1978) 1979. Figure s 2u, v, and 4o

≡ *Cercospora eriobotryicola* J.M. Yen, Bull. Soc. Mycol. France 93: 151, 1977.

≡ *Cercoseptoria eriobotryicola* (J.M. Yen) J.M. Yen, Bull. Soc. Mycol. France 97: 92, 1981.

Description: *Leaf spots* pale brown to brown with purplish brown border, circular to irregular, later confluent, 2–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal, branched, 2.5 μm wide, septate, pale brown to brown. *Stromata* well developed, amphigenous, epidermal, erumpent, substomatal, brown to olivaceous brown, 30–63 μm diam. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, simple, straight to mildly geniculate caused by sympodial proliferation, pale olivaceous to pale olivaceous brown, smooth, thin-walled, 0–2-septate, 12–33 × 2.5–3 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with indistinct and unthickened loci at the apex and shoulders caused by sympodial proliferation, 1–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, straight to mildly curved, cylindrical, smooth, thin-walled, truncate at the base, rounded to acutely rounded at the apex, 0–4-septate, 10–35 × 2–3 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Host: On leaves of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae).

Type: Taiwan, Taichung, garden of provincial agency, on leaves of *Eriobotrya japonica*, 29 Oct. 1971, J.M. Yen (PC, hb. Yen no.71266).

Material examined: **Taiwan**, Taichung, National Science Museum, on leaves of *Eriobotrya japonica*, 8 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (epitype **designated here** TUA12 = NCHUPP 3201, MycoBank MBT372258; ex-epitype culture NCHUPP L1601).

Notes: *Pseudocercospora eriobotryicola*, which has somewhat pigmented and smaller conidia, was recorded as *Cercospora eriobotryicola* on *Eriobotrya japonica* from Taiwan (Yen 1979). The symptoms and morphological characteristics of the topotype specimen of *Ps. eriobotryicola*, which was collected in this study, were identical to that of the protolog and were easily distinguishable from that of *Pseudocercospora eriobotryae*. Since the location of the type specimen deposited by Yen has been unknown, we designate the original illustration as lectotype to facilitate epitypification. The specimen (NCHUPP 3201) and its culture (NCHUPP L1601) were appropriate materials to serve as the epitype for further studies.

Pseudocercospora eupatorii-formosani U. Braun & Bagyan., Sydowia 51: 8, 1999. Figures 2w, x, and 4p

≡ *Cercospora eupatorii-formosani* Sawada, Rept. Dept, Agric. Gov. Res. Inst. Formosa 86: 169, 1943.

≡ *Pseudocercospora eupatorii-formosani* (Sawada) J.M. Yen, Gdn's Bull. Singapore 33: 175, 1980.

≡ *Pseudocercospora eupatorii-formosanae* (Sawada ex Y.L. Guo & W.H. Hsieh) J.M. Yen ex Y.L. Guo & W.H. Hsieh, Mycosystema 2: 67, 1995.

Description: *Leaf spots* reddish brown to blackish brown, angular to irregular, often confluent, 5–30 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2 μm wide, septate, subhyaline to pale olivaceous brown, thin-walled, smooth. *Stromata* amphigenous,

small composed of a few cells, or well developed, epidermal, erumpent, stomatal, pale brown to brown, to 55 μm diam, often with external hyphae. *Conidiophores* solitary from external hyphae, or loosely to densely fasciculate, arising from stromata, pale olivaceous to pale olivaceous brown, simple, straight or sinuous, smooth, thin-walled, 0–1-septate, 10–17 × 2–4 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with conically truncate and unthickened loci at the apex, 1.5–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, cylindrical to obclavate, straight to mildly curved, smooth, thin-walled, short- to long-obconically truncate at the base, rounded at the apex, 3–8-septate, 25–67 × 2–4 μm, with unthickened and not darkened hilum, 1.5–2 μm diam.

Host: On leaves of *Eupatorium* sp. (Aseteraceae).

Type: Taiwan, Taipei, on leaves of *Eupatorium formosanum* Hayata, 19 Oct. 1919, E. Kurosawa (lectotype **designated here** NTU-PPE, hb. Sawada, MycoBank MBT372657)

Material examined: **Taiwan**, Taichung, Dakeng, on leaves of *Eupatorium* sp., 9 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (epitype **designated here** TUA59 = NCHUPP 3206, MycoBank MBT372259; ex-epitype culture NCHUPP L1606).

Notes: We designate a syntype as lectotype to facilitate epitypification. Although the specimen examined in this study showed characteristics similar to those of *Cercospora eupatorii-formosani* described by Sawada, it had somewhat smaller caespituli than described in other records (Sawada 1943: caespituli 30–32 μm, conidiophores 20–44 × 3–4.5 μm, conidia 49–86 × 4.5–5 μm, 7–11-septate; Guo and Hsieh 1995: stromata 25–50 μm, conidiophores 8.5–60 × 3–4 μm, conidia 30–80 × 2.5–4 μm, 3–10-septate; Bagyanarayana and Braun 1999: stromata 10–50 μm, conidiophores 5–30 × 2–4 μm, conidia 30–80 × 1.5–4 μm, 1–8-septate). Interestingly, the concatenated sequence of this culture, consisting of ITS, *actA*, and *tef1* sequences, was almost identical to that of the ex-holotype of *Pseudocercospora eupatoriella*, which den Breeÿen et al. (2006) described as a similar fungus without external hyphae from Jamaica (6 bp differences among 662 bp). Further studies are required to confirm the taxonomy of *Pseudocercospora* species found on *Eupatorium*.

Pseudocercospora fraxinites (Ellis & Everh.) Y.L. Guo & X.J. Liu, Acta Mycol. Sin. 11: 131, 1992. Figure. 3a, b

≡ *Cercospora fraxinites* Ellis & Everh., J. Mycol. 3: 20, 1887.

Description: *Leaf spots* pale brown to greyish brown with dark brown border on the upper surface, pale brown to brown on the lower surface, angular to irregular, later confluent, 2–20 mm. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 1.5–2 μm wide, septate, hyaline to pale olivaceous brown, thin- to somewhat thick-walled, smooth. hyaline to brown. *Stromata* lacking or small, composed of a few cells, amphigenous, epidermal, erumpent, stomatal, pale brown to brown, to 25 μm diam, often with external hyphae. *Conidiophores* solitary from internal hyphae or well-developed external hyphae, or loosely to densely fasciculate, arising from stromata, simple, straight or sinuous, smooth, thin- to somewhat thick-walled, irregular in width, 16–51 × 1.5–4 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with indistinct, unthickened and truncate loci, 1–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, cylindrical to obclavate, straight to mildly curved, smooth, thin- to somewhat thick-walled, short-obconically truncate at the base, rounded at the apex, 35–80 × 2–3 μm, 6–10-septate, with unthickened and not darkened hilum, 1–2 μm diam.

Host: On leaves of *Fraxinus formosana* Hayata (Oleaceae).

Material examined: **Taiwan**, Taichung, Hsuehshan Forest Recreation Area, on leaves of *Fraxinus formosana*, 9 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (TUA71 = NCHUPP 3207; culture NCHUPP L1607).

Notes: Based on morphological characteristics, the Taiwanese specimen was considered a variation of *Pseudocercospora fraxinites*. In addition to the Taiwanese isolate, several isolates of *Ps. fraxinites* from Japan and Korea have been examined (Crous et al. 2013). However, not all isolates were found to belong to the same species, based on the phylogeny (data not shown). Further studies using North American material are required to reveal the relationships among these fungi.

Pseudocercospora fukuokaensis (Chupp) X.J. Liu & Y.L. Guo, Mycosystema 5: 103, 1992.

≡ *Cercospora fukuokaensis* Chupp, Sci. Rep. Yokahama Natl. Univ., Sect. 2, Biol. Sci. 1: 2, 1952.

Description: *Leaf spots* reddish brown to greyish brown, with dark brown border, subcircular, angular to irregular, vein limited, later confluent, 2–5 mm diam. *Caespituli* amphigenous. *Mycelium* internal, hyaline to brown, branched, 2–3 μm wide, smooth, thin-walled. *Stromata* amphigenous, small, composed of few cells on the lower leaf surface, moderately developed on the upper leaf surface, epidermal, erumpent, stomatal, pale brown to brown, pale brown to brown, 20–25 μm diam. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, or solitary to loosely fasciculate from substomatal hyphal cells, subhyaline to pale brown, simple or branched, straight to sinuous, smooth, thin-walled, 7–34 × 2–3 μm. *Conidiogenous* cells integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and truncate loci, 1–1.5 μm diam. *Conidia* solitary, holoblastic, rarely bearing microcyclic conidia, hyaline to pale olivaceous, cylindrical

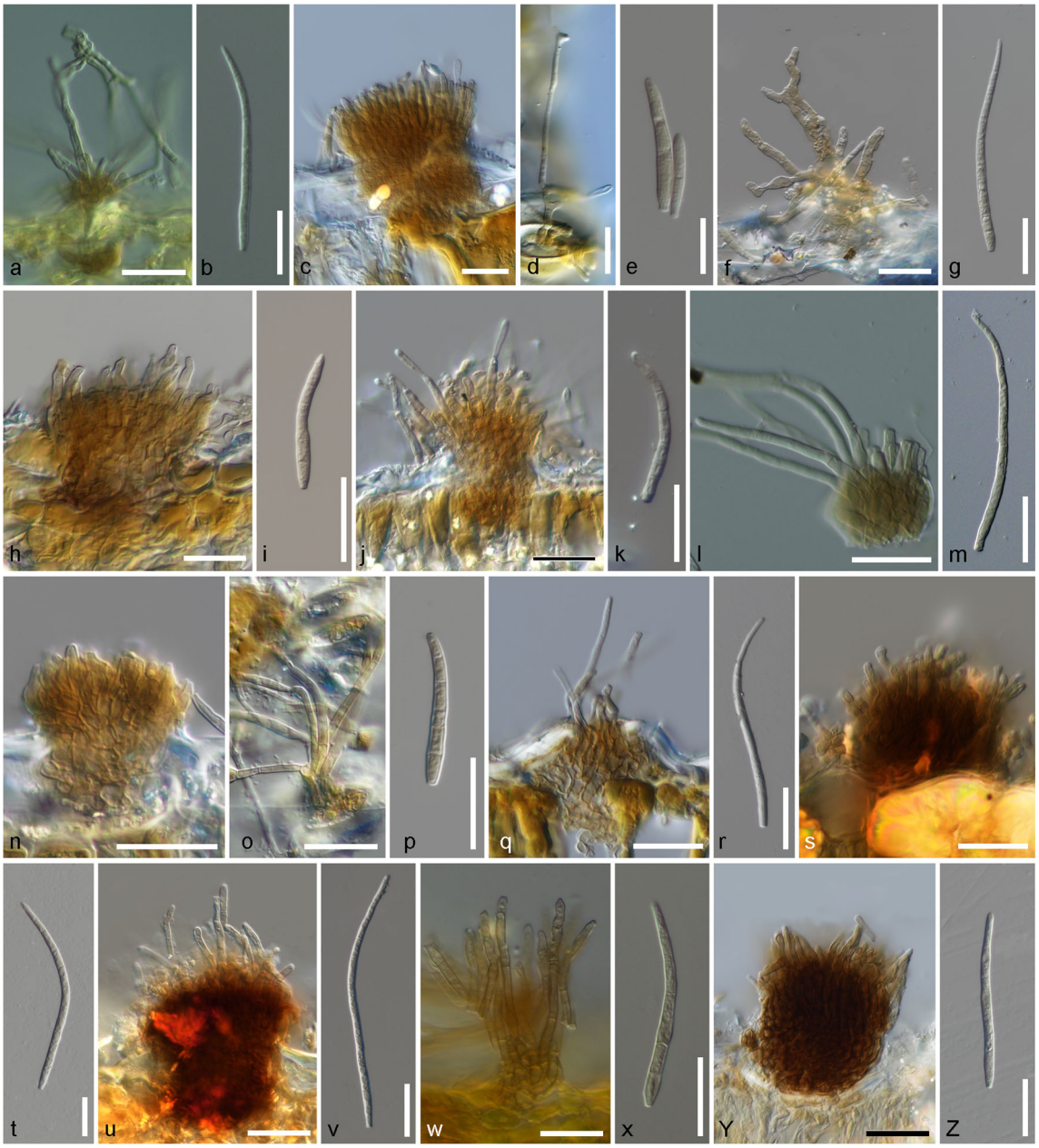

Fig.3 *Pseudocercospora fraxinites*. **a** Stroma and conidiophores. **b** Conidium. *Ps. hiratsukana*. **c** Stroma and conidiophores. **d** External hyphae with conidiophore. **e** Conidium. *Ps. houttuyniae*. **f** Conidiophroes. **g** Conidium. *Ps. humuli*. **h** Stroma and conidiophores. **i** Conidium. *Ps. imazekii*. **j** Stroma and conidiophores. **k** Conidium. *Ps. kadsurae*. **l** Stroma and conidiophores. **m** Conidium. *Ps. lonicericola*. **n** Stroma and conidiophores. **o** Creeping-conidiophores. **p** Conidium. *Ps. lyoniae*. **q** Stroma and conidiophores. **r** Conidium. *Ps. naitoi*. **s** Stroma and conidiophores. **t** Conidium. *Ps. nandinae*. **u** Stroma and conidiophores. **v** Conidium. *Ps. pouzolziae*. **w** Stroma and conidiophores. **x** Conidium. *Ps. punicae*. **y** Stroma and conidiophores. **z** Conidium. *Scale bars* 20 μm

to long obclavate, straight to sinuous, smooth, thin-walled, truncate to obconically truncate at the base, acutely rounded to rounded at the apex, 3–8-euseptate, 25–74 × 2.5–4 μm, with unthickened and not darkened hilum, 1–1.5 μm diam.

Hosts: On leaves of *Styrax japonicus* Siebold & Zucc., *S. japonicus* var. *kotoensis* (Hayata) Masam. & T.Suzuki, *S. obassia* Siebold & Zucc. (Styracaceae).

Type (examined): Japan, Fukuoka, Futsukaichi-machi, on leaves of *Styrax japonicus*, 5 Sept. 1951, S. Katsuki (holotype TNS-F-243813).

Materials examined: **Japan**, Ibaraki, on leaved of *S. japonicus*, 11 Sept. 1998, T. Kobayashi & C. Nakashima (epitype designated in Crous et al. (2013), TFM:FPH-8096; ex-epitype culture MUCC887 = MAFF237768).

Note: See Crous et al. (2013).

Pseudocercospora hachijokibushi C. Nakash., H. Horie & Tak. Kobay., Mycoscience 45: 50, 2004.

Description: See Nakashima et al. (2004).

Host: On leaves of *Stachyurus praecox* Siebold & Zucc. var. *matsuzakii* (Nakai) Makino ex H.Hara. (Stachyuraceae).

Type (examined): Japan, Tokyo, Ohshima Is., Senzu, on leaves of *Stachyurus praecox* var. *matsuzakii*, 26 Oct. 2001, H. Horie (holotype TFM:FPH-7619; ex-holotype culture MAFF238479 = MUCC1337).

Pseudocercospora hiratsukana (Togashi & Katsuki) Deighton, Mycol. Pap. 140: 34, 1976. Figure 3c, d, e

≡ *Cercospora hiratsukana* Togashi & Katsuki, J. Jpn. Bot. 28: 286. 1953.

Description: See Braun et al. (2014)

Hosts: On leaves of *Dioscorea tokoro* Makino, *Dioscorea* sp. (Dioscoreaceae).

Neotype: Japan, Kagoshima, Yaku Island, on leaves on *Dioscorea quinqueloba*, 5 Aug. 1951, S. Katsuki (neotype designated in Braun et al. (2014), CUP 40760)

Material examined: **Japan**, Tokyo, Inagi, Kurihira, on leaves of *Dioscorea tokoro*, 23 Oct. 1999, E. Imaizumi (epitype designated in Braun et al. (2014), TNS-F-61275; ex-epitype culture MAFF238300 = MUCC1105).

Pseudocercospora houttuyniae (Togashi & Katsuki) Y.L. Guo & W.X. Zhao, Acta Mycol. Sin. 8: 118, 1989. Figure. 3f, g

≡ *Cercospora houttuyniae* Togashi & Katsuki, Bot. Mag. (Tokyo) 65: 21, 1952.

Description: *Leaf spots* pale brown to greyish brown with olivaceous brown to reddish brown border, circular to subcircular, later confluent, 2–25 mm diam. *Caespituli* amphigenous, mainly hypophyllous. *Mycelium* internal and externa; external hyphae emerging through stomata or arising from stromata, branched, 2–3 μm wide, septate, hyaline to pale olivaceous brown, thin-walled, smooth. *Stromata* lacking or small, composed of few brown cells, epidermal, substomatal, to 35 μm diam. *Conidiophores* arising from stromata, emerging through stomata or branched from external hyphae, pale olivaceous brown, simple or branched, straight to geniculate, smooth to rough, thin- to somewhat thick-walled, 1–4-septate, 13–36 × 3–6 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, conically truncate at the apex, sporulating polyblastically, with unthickened loci, 2–2.5 μm diam. *Conidia* solitary, holoblastic, cylindrical to obclavate, pale olivaceous brown, smooth, somewhat thick-walled, truncate to obconically truncate at the base, acutely rounded at the apex, 1–8-euseptate, 10–65 × 2.5–4 μm, with unthickened and not darkened hilum, 2–2.5 μm diam.

Host: On leaves of *Houttuynia cordata* Thunb. (Saururaceae).

Type (examined): Japan, Kanagawa, Gontazaka, on leaves of *Houttuynia cordata*, 18 Sept. 1950, K. Togashi (holotype TNS-F-243809).

Material examined: **Japan**, Tokyo, Chofu, Jindai Bot. Garden, on leaves of *H. cordata*, 7 Nov. 1998, C. Nakashima & E. Imaizumi (epitype **designated here** TSU-MUCNS510, MycoBank MBT372141; ex-epitype culture MUCC1289 = MAFF238071).

Notes: The epitype specimen had conidiophores and conidia that were somewhat larger than those of the holotype (conidiophores: 10–80 × 3–5 μm; conidia: 40–120 × 3.5–4 μm). These characteristics were similar to those originally described by Togashi and Katsuki (1952).

Pseudocercospora humuli (Hori) Y.L. Guo & X.J. Liu, Acta Mycol. Sin.: 345. 1987. Figure 3h, i

≡ *Cercospora humuli* Hori, J. Agric. Soc. Korea 13(12): 34, 1918 (in Japanese)

= *Cercospora humuli-japonici* Sawada, Taiwan Agric. Rev.: 697, 1942. [type: NTU-PPE, hb. Sawada]

= *Pseudocercospora humuli-japonici* Sawada ex Goh & W.H. Hsieh, *Cercospora* and similar fungi from Taiwan: 239, 1990.

Description: *Leaf spots* pale brown to brown, vein limited, angular to irregular, later confluent, 2–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal, rarely external; external hyphae emerging through stomata or arising from stromata, branched, 2–2.5 μm wide, septate, subhyaline to pale brown, somewhat thick-walled, smooth. *Stromata* lacking or small to well developed, amphigenous, mainly hypophyllous, epidermal, erumpent, stomatal, brown, to 50 μm diam. *Conidiophores* densely fasciculate, arising from the upper part of stromata, or loosely fasciculate, emerging from substomatal hyphal cells, rarely branched from external hyphae, straight to geniculate, cylindrical, smooth, somewhat thick-walled at the base, 0–1-septate, 7–44 × 2.5–4 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and protruding, or rim-like (scar-like) loci, 1–2 μm diam. *Conidia* solitary, holoblastic, pale hyaline to pale olivaceous, cylindrical to obclavate, truncate to long obconically truncate at the base, acutely rounded to rounded at the apex, 1–6-euseptate, 15–60 × 2.5–3.5 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Hosts: On leaves of *Humulus scandens* (Lour.) Merr., *H. lupulus* L. var. *lupulus*. (Cannabaceae).

Type (examined): Japan, Tokyo, Nishigahara, on leaves of *Humulus scandens*, 28 Sept. 1915, S. Hori (holotype, NIAES C-487)

Material examined: **Japan**, Wakayama, on leaves of *H. lupulus*, 30 Oct. 2007, C. Nakashima & I. Araki (epitype

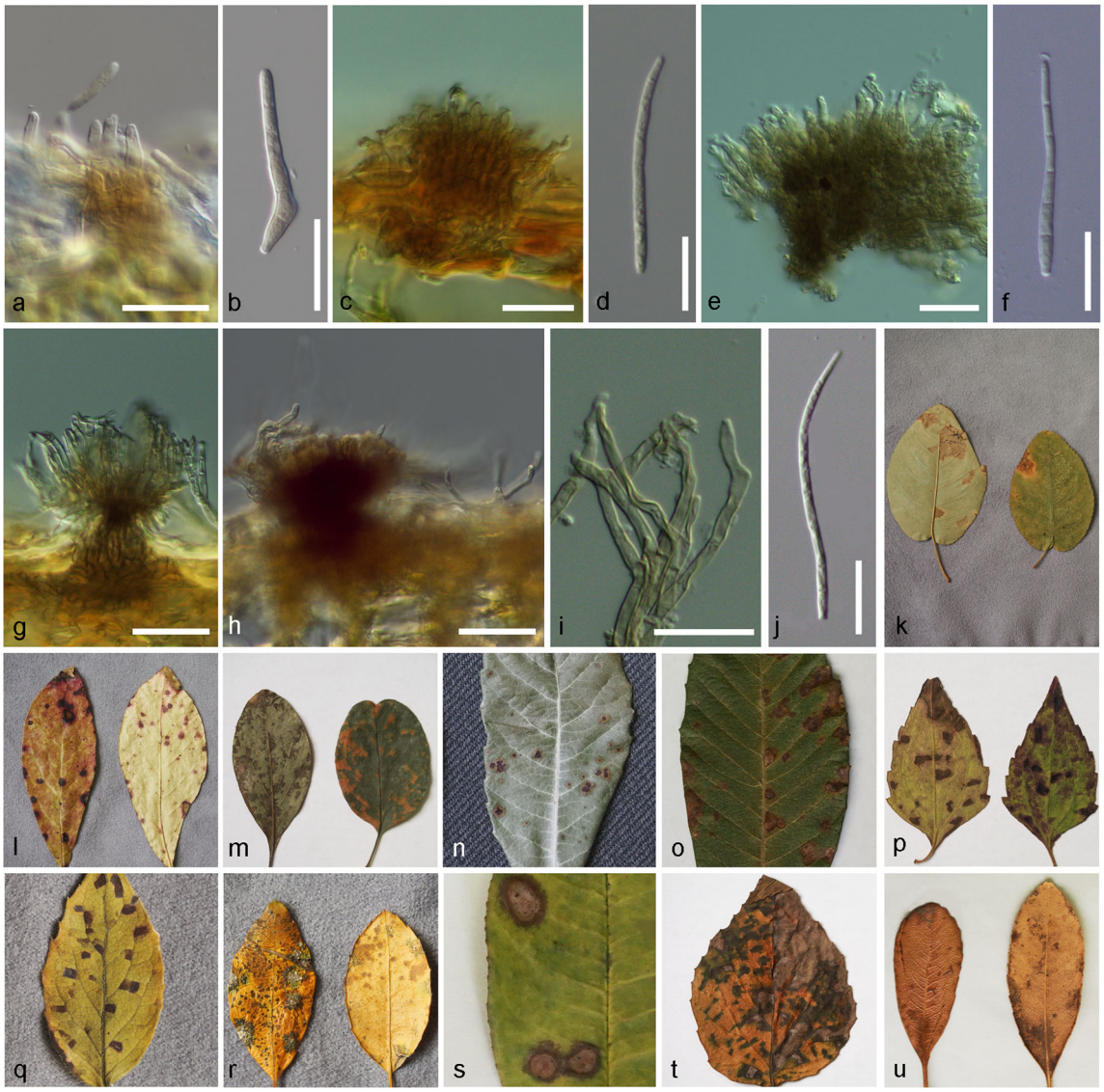

Fig. 4 *Pseudocercospora stephanandrae*. **a** Stroma and conidiophores. **b** Conidium. *Ps. tinea*. **c**. Stroma and conidiophores. **d** Conidium. *Ps. violamaculans*. **e** Stroma and conidiophores. **f** Conidium. *Ps. iwakiensis*. **g** Stroma and conidiophores. **h** Conidiophroes emerging from stroma and external hyphae. **i** Conidiophroes. **j** Conidium. Leaf spot symptoms. **k** holotype of *Ps. amelanchieris*. **l** epitype of *Ps. chibaensis*. **m** epitype of *Ps. chionanthi-retusi*. **n** *Ps. eriobotryae*. **o** epitype of *Ps. eriobotryicola*. **p** epitype of *Ps. eupatorii-formosani*. **q** epitype of *Ps. imazekii*. **r** holotype of *Ps. iwakiensis*. **s** neotype of *Ps. photiniae*. **t** epitype of *Ps. tinea*. **u** neotype of *Ps. violamaculans*. *Scale bars* 20 μm

designated in Crous et al. (2013), TFM:FPH-8097 = TSU-MUMH10867; ex-epitype culture MUCC742).

Note: See also Crous et al. (2013) for a description of this species.

Pseudocercospora imazekii Tak. Kobay. & Nagash., Trans. Mycol. Soc. Japan 32: 324, 1991. Figures 3j, k, and 4q

Description: *Leaf spots* pale brown to reddish brown on the upper surface, pale brown on the lower surface, vein limited, angular, 2–15 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2–2.5 μm wide, septate, hyaline to pale brown, thin-walled, smooth. *Stromata* amphigenous, small, composed of few brown cells, or well developed, mainly epiphyllous, brown, epidermal, erumpent, substomatal, to 48 μm diam, with external hyphae on the lower leaf surface. *Conidiophores* solitary, branched from external hyphae, or densely fasciculate, arising from the upper part of stromata, pale brown, paler towards the apex, simple, straight to geniculate caused by sympodial sporulation, smooth, thick-walled at the base, 0-4-septate, 5–40 × 2.5–4.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with truncated and unthickened loci, 1.5–3.5 μm diam. *Conidia* solitary, holoblastic, subhyaline to pale brown, obclavate to acicular, straight to slightly

curved, thin- to somewhat thick-walled, truncate to short obconically truncate at the base, rounded at the apex, 2–7-euseptate, 24–46 × 3–3.5 μm, with unthickened and not darkened hilum, 1.5–3.5 μm diam.

Host: On leaves of *Kolkwitzia amabilis* Graebn. (Caprifoliaceae).

Type (examined): Japan, Chiba Prefecture, Matsudo, on leaves of *Kolkwitzia amabilis*, 31 Oct. 1987, T. Kobayashi & M. Nagashima (holotype TFM:FPH-6913)

Material examined: **Japan**, Kanagawa Prefecture, Kamakura, on leaves of *K. amabilis*, 19 Sept. 2012, H. Horie (epitype **designated here** Hosei Univ. 12-ID0153, MycoBank MBT372142; iso-epitype TSU-MUMH11418; ex-epitype culture MUCC1668).

Pseudocercospora iwakiensis C. Nakash., K. Shibayama & Motohashi, **sp. nov**. [**MB 817645**] Fig. 4g, h, i, j, r

Etymology: Name is derived from Iwaki, the mountain from which it was collected.

Description: *Leaf spot* amphigenous, circular to irregular with distinct and dark brown border, brown, later turning to grey on the upper leaf surface, pale brown on the lower leaf surface, 2–5 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae arising from stromata, branched, 1.5–2 μm wide, septate, hyaline to pale brown, thin-walled, smooth. *Stromata* well developed, amphigenous, epidermal, erumpent, substomatal, pale brown to dark brown, 40–150(−250) μm diam, with external hyphae on the lower leaf surface. *Conidiophores* densely fasciculate, arising from stromata, often sporodochial, or solitary from external hyphae, subhyaline to dark brown, paler towards the apex, simple, rarely branched, straight to sinuous, smooth, thin-walled, 0–4-septate, 12.5–150 × 1.5–5 μm. *Conidiogenous cells* integrated, terminal or intercalary, proliferating sympodially, sporulating polyblastically, with unthickened loci, 2–2.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, cylindrical to long obclavate, straight to curved, smooth, thin-walled, obconically truncate at the base, acutely rounded at the apex, 5–14-euseptate, 30–125 × 2–2.5 μm, with unthickened and not darkened hilum, 2–2.5 μm diam.

Host: On leaves of *Ilex crenata* Thunb. (Aquifoliaceae).

Material examined: **Japan**, Aomori, Mt. Iwaki, on leaves of *Ilex crenata*, 8 Jul. 2014, C. Nakashima, K. Shibayama & K. Motohashi (holotype TSU-MUMH11502; ex-holotype culture MUCC1736).

Notes: On the plant genus *Ilex*, five *Pseudocercospora* species are hitherto known. These are *Pseudocercospora ilicis*, *Ps. ilicis-micrococcae*, *Ps. mate*, *Ps. naitoi*, and *Ps. pulvinula*. Newly described species *Ps. iwakiensis* is characterized mainly by the external hyphae, long and narrow conidia, and densely fasciculate conidiophores on well-developed stromata compared to other *Pseudocercospora* spp. on *Ilex*.

Key to Pseudocercospora species on Ilex spp.

1 Mycelium internal.....2

1* Mycelium internal and external.....4

2 Caespituli epiphyllous; Stromata moderate to well developed, up to 150 μm diam.

.....*Ps. mate*

2* Caespituli amphigenous; Stromata small, up to 45 μm diam.

3 Conidiophores simple, straight; Conidia subhyaline to olivaceous

.....*Ps.ilicis*

3* Conidiophores simple and branched, tortuous; Conidia olivaceous

.....*Ps. ilicis-micrococcae*

4 Stromata small, up to 26 μm diam.

.....*Ps. naitoi*

4* Stromata well developed, 30–150(−250) μm diam. 5

5 Conidiophores simple, 5–50(−75) × 2–5 μm; Conidia subhyaline to pale olivaceous, 20–100 × 2–4 μm

Ps. pulvinula

5* Conidiophores simple and branched, 12–150 × 1.5–5 μm; Conidia hyaline, 30–125 × 2–2.5 μm

Ps. iwakiensis

Pseudocercospora izuohshimensis C. Nakash., H. Horie & Tak. Kobay., Mycoscience 45: 49, 2004.

Description and illustration: See Nakashima et al. (2004).

Host: On leaves of *Helwingia japonica* (Thunb.) F.Dietr. (Helwingiaceae).

Type (examined): Japan, Tokyo, Ohshima Is., Senzu, on leaves of *Helwingia japonica*, 24 Sept. 2001, H. Horie (holotype TFM:FPH-7618; ex-holotype culture MAFF238478 = MUCC1336).

Pseudocercospora kadsurae (Togashi & Katsuki) Y.L. Guo & X.J. Liu, Mycosystema 5: 104, 1992. Figure 3l, m

≡ *Cercospora kadsurae* Togashi & Katsuki, Bot. Mag. (Tokyo) 65: 22, 1952.

Description: *Leaf spots* pale brown to greyish brown with brown border, circular to subcircular, 3–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal, branched, 3 μm wide, septate, hyaline to pale olivaceous brown, smooth, thin-walled. *Stromata* small, composed of several brown cells, or well developed, amphigenous, stomatal, epidermal, erumpent, substomatal, pale olivaceous to brown, to 75 μm diam. *Conidiophores* loosely to densely fasciculate, arising from the upper part of stromata, subhyaline to pale brown, paler towards the apex, simple, cylindrical, straight to slightly sinuous, smooth, thin-walled, 0–2-septate, 12.5–45 × 3–5 μm. *Conidiogenous cells* integrated, terminal, proliferating

sympodially or percurrently, sporulating polyblastically, with rim-like or truncate and unthickened loci at the shoulders and apex, 2.5–5 μm diam. *Conidia* solitary, holoblastic, subhyaline to pale brown, mainly long-cylindrical to filiform, straight to curved, smooth, thin-walled, truncate at the base, rounded at the apex, thin-walled, mostly regular in width, 40–130 × 2.5–5 μm, indistinctly 0–11-euseptate, with unthickened and not darkened hilum, 2.5–5 μm diam,

Host: On leaves of *Kadsura japonica* (L.) Dunal (Schisandraceae).

Type (examined): Japan, Kagoshima Prefecture, Yakushima Is., on leaves of *Kadsura japonica*, 19 Oct. 1949, S. Katsuki (holotype TNS-F-243807).

Material examined: **Japan**, Aichi Prefecture, Nagoya, Higashiyama Botanical Garden, on leaves of *K. japonica*, 31 Oct. 2012, C. Nakashima, K. Motohashi & I.Araki (epitype **designated here** TSU-MUMH10872, MycoBank MBT372143; ex-epitype culture MUCC752).

Notes: *Pseudocercospora kadsurae* was reassigned from the genus *Cercospora* by Guo and Liu (1992), based on a fungus on *Magnolia officinalis* subsp. *biloba* (Magnoliaceae). Although most of the conidiophores and conidia of the holotype specimen examined in this study had been depleted, its morphological characteristics confirmed that this fungus should be placed in the genus *Pseudocercospora*. However, the host genus *Kadsura* is presently associated with the family *Schisandraceae*. From the description and line drawings by Guo and Hsieh (1995), *Pseudocercospora* species on *Magnolia*, which have well-developed stromata and short conidiophores probably represent species that are distinct from *Ps. kadsurae*.

Pseudocercospora lonicericola (W. Yamam.) Deighton, Mycol. Pap. 140: 146, 1976. Figure 3n, o, p

≡ *Cercospora lonicericola* W. Yamam., J. Soc. Trop. Agric. 6: 604, 1934.

Description: *Leaf spots* reddish brown to brown on the upper surface, pale brown on the lower surface, subcircular to irregular, 2–10 mm diam. *Caespituli* amphigenous, mainly hypophyllous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 1.5–2 μm wide, septate, hyaline to pale brown, somewhat thick-walled, smooth to rough. *Stromata* lacking to small, composed of several brown cells, stomatal, with external hyphae, or well developed, epidermal, erumpent, substomatal, globose, to 48 μm diam. *Conidiophores* loosely fasciculate, emerging from substomatal hyphal cells, densely fasciculate, arising from the upper part of stromata, or solitary from external hyphae, subhyaline to pale brown, simple or branched, straight to geniculate due to sympodial sporulation, smooth, somewhat thick-walled, 0–5-septate, 6–40 × 2–2.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with rim-like or truncate and unthickened loci at the shoulders and apex, 1–2 μm diam. *Conidia* solitary, holoblastic, subhyaline to pale brown, cylindrical to obclavate, straight to curved, smooth to rough, thick-walled, obconically truncate at the base, rounded at the apex, 2–6-euseptate 25–64 × 2.5–5 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Hosts: On leaves of *Lonicera japonica* Thunb., *L. gracilipes* var. *glabra* Miq. (Caprifoliaceae).

Type: Taiwan, Taipei, on leaves of *Lonicera japonica* var. *sempervillosa* (= *Lonicera japonica*), 3 Nov. 1933, W. Yamamoto (holotype NTU-PPE, hb. Sawada).

Material examined: **Japan**, Ibaraki, on leaves of *L. gracilipes* var. *glabra* Miq., 11 Sep. 1998, T. Kobayashi (epitype **designate here** TFM:FPH-8098, MycoBank MBT372262; ex-epitype culture MUCC889 = MAFF237785)

Notes: The holotype of *Ps. lonicericola* was rediscovered in NTU-PPE. Therefore, the status of the neotype, selected in Crous et al. (2013), has been changed to epitype.

Pseudocercospora lyoniae (Katsuki & Tak. Kobay.) Deighton, Trans. Brit. Mycol. Soc. 88: 389, 1987. Figure 3q, r

≡ *Cercospora lyoniae* Katsuki & Tak. Kobay., Trans. Mycol. Soc. Japan 16: 3, 1975.

Description: *Leaf spots* pale brown to brown on the upper surface, pale brown on the lower leaf surface, vein limited, angular to irregular, later confluent, 2–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2–2.5 μm wide, septate, hyaline to pale olivaceous brown, thin-walled, smooth to rough. *Stromata* lacking or small, composed of few cells on the lower leaf surface, stomatal, small to moderate on the upper leaf surface, epidermal, erumpent, stomatal, pale brown to brown, 15–41 μm diam, often with external hyphae. *Conidiophores* loosely fasciculate, arising from stromata or emerging through stomata, or solitary from external hyphae, subhyaline to pale brown, paler towards the apex, simple, sinuous to geniculate, smooth, thin-walled, 0–1-septate, 16–51 × 2.5–3 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and truncate loci, 1.5–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale brown, smooth, filiform to long obclavate, thin- to somewhat thick-walled, truncate at the base, acutely rounded at the apex, 5–8-euseptate, 40–88 × 2.5–3.5 μm, with unthickened and not darkened hilum, 1.5–2 μm diam.

Host: On leaves of *Lyonia ovalifolia* (Wall.) Drude var. *elliptica* (Siebold & Zucc.) Hand.-Mazz. (Ericaceae).

Type (examined): Japan, Tokyo, Aasakawa, on leaves of *Lyonia ovalifolia* var. *elliptica*, 21 Sep. 1973, H. Horie (holotype TFM:FPH-3999)

Material examined: **Japan**, Chofu, Jindai Bot. Gard., on leaves of *L. ovalifolia* var. *elliptica*, 7 Nov. 1998, C. Nakashima & E. Imaizumi (epitype designated in Crous et al. (2013), TFM:FPH-8100; ex-epitype culture MUCC910 = MAFF237775).

Pseudocercospora naitoi (Togashi) C. Nakash. & Tak. Kobay., Mycoscience 40: 272, 1999. Figure 3s, t

≡ *Cercospora naitoi* Togashi, Trans. Sapporo Nat. Hist. Soc. 17: 101, 1942.

= *Cercospora mate* sensu Naito, Mem. Coll. Agric. Kyoto Imp. Univ. 47: 49, 1940, non *Cercosporina mate* Speg.

Description: *Leaf spots* reddish brown to dark brown on the upper surface, grey to greyish brown on the lower surface, vein limited, angular to irregular, confluent, 3–10 mm. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2 μm wide, septate, hyaline to pale olivaceous brown, thin- to somewhat thick-walled, smooth. *Stromata* lacking or small on the lower surface, to 26 μm diam, small to well developed on the upper surface, 23–60 μm diam, brown, epidermal, erumpent, stomatal, with external hyphae. *Conidiophores* loosely to densely fasciculate, emerging from the upper part of stromata, or solitary from external hyphae, pale brown to brown, paler towards the apex, simple, straight o geniculate, smooth, thin- to somewhat thick-walled, 0–1-septate, 6–20 × 2–3 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with indistinct and unthickened loci, 1.5–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, cylindrical to acicular, straight to curved, smooth, thin- to somewhat thick-walled, truncate to obconically truncate at the base, acutely rounded to rounded at the apex, 3–10-euseptate, 40–90 × 1.5–3 μm, with unthickened and not darkened hilum, 1.5–2 μm diam.

Hosts: On leaves of *Ilex chinensis* Sims (= *Ilex oldhamii* Miq.), *I. serrata* Thunb., *I. serrata* Thunb. f. *argutidens* (Miq.) Sa.Kurata (Aquifoliaceae).

Type (examined): Japan, Kyoto, Shogun-zizo, on leaves of *Ilex chinensis*, 20 Sept. 1924, T. Nojima (holotype TNS-F-243817).

Material examined: **Japan**, Shizuoka Prefecture, Hamakita, on leaves of *I. serrata* f. *argutidens*, 1 Nov. 1997, T. Kobayashi & C. Nakashima (epitype **designated here** TSU-MUCNS142, MycoBank MBT372148; ex-epitype culture MUCC1072 = MAFF237906).

Notes: This fungus was reported from Japan as "*Cercospora mate* Speg." by Naito (1942). However, the name in the genus *Cercospora* did not exist at that time. Judging from the original description, Naito simply misapplied the name to *Cercosporina mate*, without meaning to propose a new combination. Later, Togashi (1942) re-examined several specimens that were examined by Naito. From his own observations, Togashi proposed a new species as *Cercospora naitoi* based on a newly collected specimen, which was different from the specimens examined by Naito (1942). Thus, the nomenclatural types of the two species, *Cercospora naitoi* and *Cercospora mate* sensu Naito, are different.

Pseudocercospora nandinae (Nagat.) X.J. Liu & Y.L. Guo, Acta Mycol. Sin. 11: 297, 1992. Figure 3u, v

≡ *Cercospora nandinae* Nagat., Materials for Education, Kyoto Pref. 3: 79, 1932; Forsch. Pflanzenkrankh. Univ. Kyoto. 3: 111, 1937, nom. illegit.

= *Cercospora nandinae* Fukui, Bull. Imp. Coll. Agric. Mie: 14, 1933, nom. illegit.

Description: *Leaf spots* dark purplish brown to blackish brown with red border, irregular, confluent, 2–30 mm. *Caespituli* hypophyllous. *Mycelium* internal, branched, 2–3 μm wide, septate, hyaline to pale brown, thin- to thick-walled, smooth to rough. *Stromata* hypophyllous, small to well developed, epidermal, erumpent, substomatal, brown, 47–85 μm diam. *Conidiophores* densely fasciculate, arising from stromata, or solitary, emerging from substomatal hyphal cells, subhyaline to pale brown, paler towards the apex, simple or branched, straight to geniculate, smooth to rough, thin- to thick-walled, 0–5-septate, 25–78 × 2.5–4.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and indistinct conidial loci, 2–2.3 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, acicular, cylindrical to obclavate, smooth, thin- to somewhat thick-walled, obconically truncate at the base, acutely rounded at the apex, 2–12-euseptate, 40–130 × 2.5–4.5 μm, with unthickened and not darkened hilum, 2–2.5 μm diam.

Host: On leaves of *Nandina domestica* Thunb. (Berberidaceae).

Type: Japan, Kumamoto Prefecture, on leaves of *Nandina domestica*, 7 Sept. 1926, I. Nagatomo (KOU: Herbarium, laboratory of plant pathology, Kyoto University, Kyoto, Japan).

Material examined: **Japan**, Shizuoka Prefecture, Hamakita, on leaves of *Nandina domestica*, 6 Nov. 1996, T. Kobayashi & C. Nakashima (epitype **designated here** TSU-MUCNS129, MycoBank MBT372149; ex-epitype culture MUCC1260 = MAFF239633).

Pseudocercospora paederiae Goh & W.H. Hsieh, *Cercospora* and similar fungi from Taiwan: 291, 1990.

≡ *Cercospora paederiae* Sawada, Rep. Gov. Agric. Res. Inst. Taiwan 87: 84, 1944, nom. illegit.

≡ *Pseudocercospora paedriicola* C. Nakash. & Tak. Kobay., J. Agric. Sci., Tokyo Univ. of Agric. 48: 98, 2003, nom. superfl.

= *Cercospora paederiae* F.L. Tai, Bull. Chinese Bot. Soc.: 56, 1936.

Description and illustration: See Hsieh and Goh (1995).

Host: On leaves of *Paederia foetida* L. (Rubiaceae).

Type: Taiwan, Taipei, Tshau-suann, on leaves of *Paederia scandens* (= *Paederia foetida*), 12 Oct. 1912, E. Kurosawa (holotype NTU-PPE, hb. Sawada).

Material examined: **Japan**, Kagoshima Prefecture, Okinoerabu Is., on leaves of *Paederia foetida*, Nov. 2001, Y. Ono (culture MUCC1355 = MAFF239161).

Note: Hsieh and Goh (1990) cited that the collector of holotype was K. Sawada. However, to be exact, it is E. Kurosawa.

Pseudocercospora photiniae (Fukui) C. Nakash. & Tak. Kobay., Mycoscience 41: 25, 2000. Figure 4s

≡ *Cercospora photiniae* Fukui, Bull. Mie Imp. Agric. Forest. 3: 12, 1933.

Description: *Leaf spots* greyish brown at the centre, with reddish brown to purplish brown border, often confluent, circular, 1–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2 μm wide, septate, subhyaline to pale brown, thin-walled, smooth. *Stromata* amphigenous, small, composed of several cells, or moderately developed, epidermal, erumpent, stomatal, pale brown to brown, to 63 μm diam. *Conidiophores* loosely fasciculate arising from stromata, subhyaline to pale brown, simple, straight to geniculate, smooth, 0–3-septate, 15–30 × 2–3 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and truncate loci, 2 μm diam. *Conidia* solitary, holoblastic, pale olivaceous to pale olivaceous brown, cylindrical to obclavate, straight to mildly curved, smooth, thin-walled, long-obconically truncate to truncate at the base, acutely rounded at the apex, 3–9-septate, 30–80 × 2–3 μm, with unthickened and not darkened hilum, 2 μm diam.

Host: On leaves of *Photinia glabra* (Thunb.) Maxim. (Rosaceae).

Type: Japan, Mie Prefecture, Kurima, Imperial College of Agr. & Forst. Mie, on leaves of *Photinia glabra*, 20 September 1932, T. Fukui (not preserved).

Material examined: **Japan**, Mie Prefecture, Tsu, Mie University, on leaves of *Photinia glabra*, 31 Oct. 2012, C. Nakashima, S. Ito & K. Kimura (neotype **designated here** TSU-MUMH11410, MycoBank MBT372150; ex-neotype culture MUCC1661).

Note: The neotype specimen is topotype material from the site where the original specimen was collected by T. Fukui. See Nakashima and Kobayashi (2000) for notes and illustrations.

Pseudocercospora pouzolziae (Syd.) Y.L. Guo & X.J. Liu, Acta Mycol. Sin.: 298, 1992. Figure 3w, x

≡ *Cercospora pouzolziae* Syd., Ann. Mycol. 33: 236, 1935.

Description: *Leaf spots* greyish brown at the centre with dark brown border on the upper surface, brown on the lower surface, vein limited, later confluent, angular to irregular, 1–5 mm diam. *Caespituli* amphigenous. *Mycelium* internal, pale brown to brown. *Stromata* amphigenous, small to well developed, epidermal, erumpent, stomatal, pale brown to brown, to 50 μm diam. *Conidiophores* loosely fasciculate arising from stromata, simple, straight in the lower part, geniculate in the upper part, smooth to rough, pale brown to brown, paler towards the apex, irregular in width in the upper part, 30–80 × 2.5–4 μm, 0–3-septate. *Conidiogenous cells* integrated, terminal, proliferating sympodially, sporulating polyblastically, with unthickened and obconically truncate loci, 1–2 μm diam. *Conidia* solitary, holoblastic, pale olivaceous to pale brown, smooth, straight to mildly curved, cylindrical to obclavate, short-obconically truncate and unthickened at the base, 1–2 μm diam, rounded at the apex, 30–88 × 2.5–5 μm, 3–8-septate.

Host: On leaves of *Pouzolzia* sp. (Urticaceae).

Type: South Africa, Transvaal, Barberton, Nelspruit, on leaves of *Pouzolzia hypoleuca*, May 1931, L.C.C. Liebenberg (lectotype **designated here** PREM 26013, MycoBank MBT372673; iso-lectotypes CUP-040633, 040634).

Material examined: **Taiwan**, Taichung, Hsuehshan Forest Recreation Area, on leaves of *Pouzolzia* sp., 9 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (TUA80 = NCHUPP 3208; culture NCHUPP L1608).

Notes: Recently, a specimen of *Cercospora pouzolziae* (PREM 26013), which was recognized as holotype, was re-examined by Crous and Braun (1996) and it was transferred to the genus *Pseudocercospora*. However, two isotype specimens were found in CUP. Then, we designate an isotype as lectotype. *Pseudocercospora mysorensis* (= *Cercospora pouzolziae* Sawada, type in NTU-PPE hb. Sawada), another *Pseudocercospora* species on the same host, was recorded from Taiwan. This species differs from *Ps. pouzolziae* in that the latter has narrower and shorter conidiophores and conidia, and does not form well-developed external hyphae. *Pseudocercospora pouzolziae* was originally described from South Africa. Comparative studies based on morphological characteristics and phylogenetic analyses of Taiwanese collections and isolates from South Africa are required.

Pseudocercospora punicae (Henn.) Deighton, Mycol. Pap. 140: 151, 1976. Figure 3y, z

≡ *Cercospora punicae* Henn., Bot. Jahrb. Syst. 37: 165, 1906.

Description: *Leaf spots* dark brown to blackish brown on the upper surface, greyish brown, circular, angular to irregular, confluent, 2–5 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2 μm wide, septate,

hyaline to brown, thin-walled, smooth to rough. *Stromata* amphigenous, small to well-developed upper leaf surface, composed of a few brown cells to moderately developed on the lower leaf surface, epidermal, erumpent, substomatal, pale brown to dark brown, 17–80 μm diam, with external hyphae on the lower leaf surface. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, solitary from external hyphae, subhyaline to pale brown, simple or branched, straight to sinuous, smooth to rough, thick-walled, conically truncate at the apex, irregular in width, 0–5-septate, 5.5–40 × 2–4 μm. *Conidiogenous cells* integrated, terminal, proliferating percurrently, often sympodially, sporulating polyblastically, with rim-like or unthickened, protruding and truncate loci, 1.5–2.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous brown, straight to mildly curved, thin- to somewhat thick-walled, long obconically truncate at the base, acutely rounded at the apex, 20–80 × 2.5–4 μm, 3–9-euseptate, with unthickened and not darkened hilum, 1.5–2.5 μm diam.

Host: On leaves of *Punica granatum* L. (Lythraceae).

Type: Japan, Tokyo, Komaba, on leaves of *Punica granatum* L., Oct. 1904, I. Miyake (isotype S, hb. Sydow).

Materials examined: **Japan**, Shizuoka Prefecture, Hamakita, 6 Nov. 1996, T. Kobayashi & C. Nakashima (TSU-MUCNS129); **Japan**, Kagoshima, Amami-Ohshima Is., 11 Nov. 1993, T. Kobayashi & T. Hosoya (culture MUCC1209 = MAFF236998).

Notes: The collection site of the isolates examined in this study is far from the original type locality of the present species. Therefore, the designation of an epitype is deferred until a detailed examination of the topotype is completed.

Pseudocercospora* sp. on *Pyracantha

Description: *Leaf spots* brown to dark brown, with indefinite border, oval to irregular, 3–5 mm diam. *Caespituli* amphigenous, mainly epiphyllous. *Mycelium* internal and rarely external; external hyphae arising from stromata, branched, 2.5 μm wide, septate, pale brown, thin-walled, smooth. *Stromata* amphigenous, epidermal, erumpent, substomatal, pale olivaceous, globose, 30–75 μm diam, rarely with external hyphae on the lower leaf surface. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, pale brown, simple, subcylindrical to ampuliform, straight to mildly geniculate, smooth, thin-walled, 0–1-septate, 15–30 × 2.5 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with indistinct, truncate and unthickened loci at the apex and shoulders caused by sympodial proliferation, 2–2.5 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, cylindrical, smooth, straight to mildly curved, obconically truncate at the base, rounded at the apex, 0–4-septate, 26–40 × 2.5–3 μm, with unthickened and not darkened hilum, 2–2.5 μm diam.

Host: On leaves of *Pyracantha coccinea* M.Roem. (Rosaceae).

Material examined: **Taiwan**, Taichung, National Science Museum, on leaves of *Pyracantha coccinea*, 8 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (TUA31 = NCHUPP 3201; culture NCHUPP L1602).

Notes: Two *Pseudocercospora* species have been recorded on the host plant genus *Pyracantha*. Based on morphological characteristics, the sample collected from Taiwan is similar to *Pseudocercospora pyracanthigena*, except that it rarely shows external hyphae. Although we examined the type material of *Cercospora pyracanthae* deposited in TNS (TNS-F-243829: Japan, Fukuoka Prefecture, Kurume, on leaves of *Pyracantha angustifolia* (Franch.) C.K.Schneid., 6 Nov. 1947, S. Katsuki), further research is required to select an epitype for *Pseudocercospora pyracanthae*, because the type localities of *Ps. pyracanthae* and *Ps. pyracanthigena* are geographically very close. The differences in the DNA sequences of the isolates of *Ps. pyracanthae*, *Ps. pyracanthigena*, and *Pseudocercospora* sp. from Taiwan, were the following: ITS: 0/481 sites, *tef1*:39–51/311 sites, and *actA*: 0–9/198 sites.

Pseudocercospora stephanandrae (Tak. Kobay. & H. Horie) C. Nakash. & Tak. Kobay., Mycoscience 41: 27, 2000. Figure 4a, b

≡ *Cercospora stephanandrae* Tak. Kobay. & H. Horie, Trans. Mycol. Soc. Japan 20: 331, 1979.

Description: *Leaf spots* pale brown to brown on the upper surface, greyish brown to brown on the lower surface, angular to irregular, 3–10 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2.5–3 μm wide, septate, hyaline to pale olivaceous brown, thin-walled, smooth. *Stromata* lacking or small, epiphyllous, epidermal, erumpent, pale brown, 20–33 μm diam. *Conidiophores* loosely to densely fasciculate, emerging from the upper part of stromata and substomatal hyphal cells, or solitary from external hyphae on the lower surface, cylindrical, subhyaline to pale olivaceous brown, simple, straight to geniculate, smooth, thin- to somewhat thick-walled, 0–1-septate, 6–40 × 2.5–4 μm. *Conidiogenous cells* terminal, integrated, proliferating sympodially, sporulating polyblastically, often determinate, with unthickened and truncate loci or rim-like loci, 1–1.5 μm diam. *Conidia* solitary, holoblastic, hyaline to subhyaline, cylindrical to obclavate, straight to curved, smooth, short obconically truncate at the base, rounded at the apex, 2–9-euseptate, 30–65 × 1.5–4 μm, with unthickened and not darkened hilum, 1–1.5 μm diam.

Host: On leaves of *Neillia incisa* (Thunb.) S.H.Oh (= *Stephanandra incisa*) (Rosaceae).

Type (examined): Japan, Tokyo, Chofu, Jindai Bot. Gard., on leaves of *Neillia incisa*, 26 Oct. 1974, T. Kobayashi & H. Horie (holotype TFM:FPH-4411)

Materials examined: **Japan**, Tokyo, Chofu, Jindai Bot. Gard., on leaves of *Neillia incisa*, 7 Nov. 1998, C. Nakashima & E. Imaizumi (epitype designated in Crous et al. (2013), TFM:FPH-8099; ex-epitype culture MUCC914 = MAFF237799).

Pseudocercospora tinea Y.L. Guo & W.H. Hsieh, Mycosystema 7: 124, (1994) 1995. Figure 4c, d, t

Description: *Leaf spots* dark brown to purplish brown, later grey at the centre with purplish brown border on the upper surface, pale purplish brown on the lower surface, vein limited, angular to irregular, 2–5 mm diam. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 1.5–2 μm wide, septate, hyaline to pale olivaceous, thin-walled, smooth. *Stromata* small to well developed, amphigenous, mainly epiphyllous, epidermal, erumpent, substomatal, pale olivaceous brown to brown, globose, to 55 μm diam, with external hyphae. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, or solitary from external hyphae, pale olivaceous brown to pale brown, cylindrical, simple, straight or geniculate caused by sympodial proliferation, smooth, thin-walled, 0–3-septate, 10–35 × 1.5–3 μm. *Conidiogenous cells* integrated, terminal, proliferating sympodially or percurrently, sporulating polyblastically, with truncate and unthickened loci at the apex and shoulders caused by sympodial proliferation, 1–2 μm diam. *Conidia* solitary, holoblastic, hyaline to pale olivaceous, cylindrical to obclavate, straight to mildly curved, smooth, thin-walled, obconically truncate at the base, rounded at the apex, 0–7-septate, 15–52 × 2–3.5 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Host: On leaves of *Viburnum* spp. (Adoxaceae).

Type: China, Guangdong, Guangzhou, on *Viburnum macrocephalum* Fortune, 6 Sept. 1961, Q.M. Ma Liu X.J. (holotype HMAS 67254).

Materials examined: **Taiwan**, Taichung, National Science Museum, on leaves of *Viburnum* sp., 8 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (epitype **designated here** TUA40 = NCHUPP 3203, MycoBank MBT372401; ex-epitype culture NCHUPP L1603); **Taiwan**, Taichung, Dakeng, on leaves of *Viburnum* sp., 9 Oct. 2014, C. Nakashima, K. Motohashi, Y. Hattori & C.Y. Chen (TUA56 = NCHUPP 3204; culture NCHUPP L1604).

Notes: A dichotomous key to *Pseudocercospora* species on *Viburnum* was published by Braun et al. (2015b). The present species was described by Guo (1995), based on specimens from mainland China and Taiwan (Taichung), the site where we collected samples for this study. Although we could not re-examine the type material collected from mainland China (HMAS 67254) and its isolate, the morphological characteristics of the samples collected in Taichung were identical to those of the protolog of *Pseudocercospora tinea*.

Pseudocercospora violamaculans (Fukui) Tak. Kobay. & C. Nakash., Mycoscience 43: 225, 2002. Figure 4e, f, u

≡ *Cercospora violamaculans* Fukui, Bull. Mie Imp. Coll. Agric. Forest. 3:15, 1933.

Description: *Leaf spots* amphigenous, greyish brown at the centre, brown to dark brown with dark purplish brown border on the upper leaf surface, brown with dark brown boarder on the lower leaf surface, often enlarged and confluent, irregular 3–15 mm. *Caespituli* amphigenous. *Mycelium* internal and external; external hyphae emerging through stomata or arising from stromata, branched, 2–3 μm wide, septate, hyaline to subhyaline, thin-walled, smooth. *Stromata* small to well developed, amphigenous, epidermal, erumpent, substomatal, pale brown to dark brown, 38–114 μm diam. *Conidiophores* emerging from the upper part of stromata, or branched from external hyphae at the lower leaf surface, subhyaline to pale brown, straight or geniculate, simple or branched, smooth to rough, thin-walled, 0–4-euseptate, 16–40 × 1.5–4 μm. *Conidiogenous cells* integrated, terminal and intercalary, proliferating sympodially, or percurrently, sporulating polyblastically, with truncate and unthickened loci on the shoulders caused by sympodial sporulation, 1–2 μm diam. *Conidia* solitary, holoblastic, hyaline to subhyaline, cylindrical to obclavate, straight to curved, smooth to rough, thin-walled, truncate to short obconically truncate at the base, acutely rounded to rounded at the apex, often constricted at the septa, 1–5-euseptate, 16–50 × 2–3.5 μm, with unthickened and not darkened hilum, 1–2 μm diam.

Host: On leaves of *Rhaphiolepis indica* (L.) Lindl. var. *umbellata* (Thunb.) H.Ohashi (Rosaceae).

Type: Japan, Mie Prefecture, Kurima, Imperial College of Agr. & Forst. Mie, on leaves of *Rhaphiolepis indica* var. *umbellata*, 2 June 1932, T. Fukui (Not preserved).

Material examined: **Japan**, Mie Prefecture, Tsu, Kurima-machiya, Mie University, 31 Oct. 2012, C. Nakashima, S. Ito & K. Kimura (neotype **designated here** TSU-MUMH11409, MycoBank MBT372151; ex-neotype culture MUCC1660).

Note: The neotype specimen represents topotype material from the site where the original specimen was collected by T. Fukui.

Pseudocercospora xenosyzygiicola Crous, Mycol. Res. 103: 616, 1999.

≡ *Cercospora eugeniae* Sawada, Rep. Gov. Agric. Res. Inst. Taiwan 85: 104, 1943, nom. illegit.

Description and *illustration*: See Nakashima et al. (2002).

Host: On leaves of *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Myrtaceae).

Type: Taiwan, Tainan, Yujing, on leaves of *Eugenia javanica* (= *Syzygium samarangense*), 10 May 1931, K. Sawada, syntype in Sawada 1943 (lectotype **designated here** NTU-PPE, hb. Sawada, MycoBank MBT372663; isolectotype TNS-F-220420, BPI436211); Taiwan, Tainan, Singying, on leaves of *E. javanica*, 25 Nov. 1928, Sawada, syntype in Sawada 1943 (NTU-PPE, hb. Sawada).

Materials examined: **Taiwan**, Tainan, Yujing, on leaves of *Syzygium samarangense*, 10 May 1931, K. Sawada (isosyntype TNS-F-220420); **Japan**, Okinawa Prefecture, Okinawa Is., on leaves of *S. samarangense*, 7 Mar. 1998, T. Kobayashi & C. Nakashima (epitype **designated here** TSU-MUMH11540, MycoBank MBT372402; ex-epitype culture MUCC1481 = MAFF237986).

Notes: See Nakashima et al. (2002) for a description and illustration of this species. We designate the syntype as lectotype to facilitate epitypification. And also, the fresh material collected in Okinawa, Japan, was located in the same island arc as Taiwan, and thus is an adequate epitype.

Pseudocercospora zelkovae (Hori) X.J. Liu & Y.L. Guo, Acta Mycol. Sin. 12: 33, 1993.

≡ *Cercospora zelkowae* Hori, "as *zelkowa*", in Nambu, J. Pl. Protect. 8: 492, 1921.

Description: Leaf spots brown, circular to irregular, often confluent, 2–20 mm diam. *Caespituli* hypophyllous. *Mycelium* internal, branched, 2–3-μm wide, septate, hyaline to pale olivaceous, thin-walled, smooth. *Stromata* well developed, hypophyllous, epidermal, erumpent, substomatal, subhyaline to pale olivaceous brown, globose, 28–64 μm diam. *Conidiophores* densely fasciculate, emerging from the upper part of stromata, subhyaline to pale brown, simple, straight to slightly sinuous, smooth, thin-walled, 0–4-septate, 13–46 × 3–5 μm. *Conidiogenous cells* integrated, terminal, proliferating percurrently, sporulating polyblastically, with rim-like loci at the apex. *Conidia* solitary, holoblastic, hyaline to subhyaline, cylindrical to long-obclavate, rarely branched, straight to mildly curved, smooth, thin-walled, truncate to long-obconically truncate at the base, rounded to acutely rounded at the apex, 3–10-septate, 25–73 × 2.5–4 μm, with unthickened and not darkened hilum, 1.5–2.5 μm diam.

Host: On leaves of *Zelkowa serrata* Makino (Ulmaceae).

Materials examined: **Japan**, Yamagata, Kamabuchi, on leaves of *Zelkowa serrata*, 5 July 1956, K. Ito (neotype designated in Crous et al. (2013), TFM:FPH-169); **Japan**, Shizuoka Prefecture, on leaves of *Z. serrata*, 31 Oct. 1996, T. Kobayashi & C. Nakashima (TSU-MUMH11506 = MUCNS100; culture MUCC872 = MAFF238237).

Note: Sequences of the ex-neotype culture (MAFF 410008) were not included in the analyses because its *rpb2* locus could not be amplified under the conditions used for this study.

Discussion

We examined the morphological characteristics of type materials mainly from Japan and Taiwan using morphological criteria as recently defined by Braun et al. (2013) and Crous et al. (2013), and analyzed the phylogenetic relationships of these isolates. In the case of lost or destroyed type materials, neotypes and derived ex-neotype isolates were selected among appropriate collections, if available among topotype material. Species with existing old types were epitypified with appropriate collections, including corresponding cultures and sequence data. The phylogenetic tree (Fig. 1) revealed that the genus *Pseudocercospora* formed a monophyletic clade composed of two well-supported major subclades and *Pseudocercospora vitis*, which was on a basal node of the tree, as previously reported (Crous et al. 2013; Bakhshi et al. 2014; Shivas et al. 2015; Liang et al. 2016). In addition, we found that 1) each species from a different host plant genus clustered separately; 2) except for a few species, *Pseudocercospora* species were observed to be host-specific; 3) isolates of the same species had nearly identical sequences; for example, two isolates of *Pseudocercospora tinea* examined in this study showed a difference at 2/1,842 sites; and 4) *Pseudocercospora* species could be distinguished based on their morphological characteristics in vivo. These results support the characteristics of *Pseudocercospora* as defined in previous reports (Braun et al. 2013; Crous et al. 2013). Furthermore, we found that *Pseudocercospora nephrolepidicola* was distinct from the *Pseudocercospora* clade. Crous et al. (2010) noted that *Pseudocercospora nephrolepidicola* sequences were closely related to those of *Mycosphaerella quasiparkii*, which was recently reassigned as *Clypeosphaerella quasiparkii* (Guatimosim et al. 2016), suggesting that *Ps. nephrolepidicola* should also be allocated to another genus. According to Kirschner and Wang (2015), the ITS sequence of *Ps. nephrolepidicola* was closely related to that of *Passalora lygodii*. The taxonomical position of this species needs still to be studied.

By adding the *rpb2* region in addition to the concatenated ITS-*actA*-*tef1* alignment, the genetic distances among *Pseudocercospora* species (Fig. 1) were found to be better supported than in previous studies (Bakhshi et al. 2014; Shivas et al. 2015; Silva et al. 2016). Results from this study suggest that the *rpb2* locus should in the future be added as a robust molecular marker for recognition of species within the genus *Pseudocercospora*.

Although concatenated sequences from the different isolates of the same species, except for *Ps. tinea*, were not included in this analysis, those of *Ps. chibaensis*, *Ps. daphniphylli*, and *Ps. izuohshimensis*, were found to have nearly identical sequences (2 bp differences among 1842 bp) (ESM. 1). Also, *Ps. imazekii* and *Ps. fukuokaensis* have only 2 bp differences among 1,842 bp. The results of the present

analyses might suggest that they pertain to s single species. However, the host family of each of these three *Pseudocerocospora* species, as well as the collection date and place, were different. Moreover, some morphological characters, especially the shape and size of the conidiophores and conidia on each host, differed among the species. Further studies using additional gene loci are required to understand the ecological and morphological diversification of these species.

Pseudocercospora eriobotryae and *Ps. eriobotryicola* from Japan and Taiwan, respectively, were described on species of the plant genus *Eriobotrya* (Rosaceae), which is native to Asia. There are small differences in conidia and caespituli between these species. However, our phylogenetic tree suggests that these species have a common ancestor. Furthermore, several *Pseudocercospora* species on closely related plants belonging to the subfamily Amygdaloideae (Spiraeoideae) of Rosaceae (Potter et al. 2007), namely *Ps. violamaculans* on *Rhaphiolepis* and *Pseudocercospora* sp. on *Pyracantha* (NCHUPP 3201), which are also native to Asia, have speciated on Amygdaloideae plants in Asia. This might be the first clear evidence of co-speciation of *Pseudocercospora* species and their host plants.

The external mycelium of *Pseudocercospora* species often bear conidiophores, which represent a morphological feature used for species delimitation (Crous et al. 2013). The size of caespituli and external hyphae of *Pseudocercospora* species on *Eupatorium* has been used to distinguish between *Pseudocercospora eupatorii-formosani* and *Pseudocercospora eupatoriella*, which have nearly identical sequences. The value of this criterion in delimiting species on *Eupatorium* might have been overestimated. In addition, the presence or absence of external mycelium was frequently ignored by authors in the protologs of these species, especially in descriptions of species of *Cercospora* sensu lato, including *C. houttuyniae*, which has well-developed external hyphae. When preparing species descriptions for species distinct from hitherto known species or when identifying species identical to those inhabiting a disjunct area, the type specimens, including the holotype and/or epitype from topotype specimens, should be included in the analyses. On the other hand, one *Pseudocercospora* isolate (TUA29) was obtained as an epiphyte, which colonized leaves of the plant genus *Sterculia* without penetrating the plant tissue. It was located close to *Ps. eupatorii-formosani* in this phylogenetic tree (6 bp differences among 1842 bp). So far, several cases that other species can colonise non-host plant have been reported. This phenomenon of temporarily colonising a non-host while searching for its true host was coined the pogostick hypothesis by Crous and Groenewald (2005). The search for a true host plant for this isolate is interesting from the viewpoint of considering the host expansion strategy of *Pseudocercospora* spp.

At present, more than 160 *Pseudocercospora* species from Japan have been recorded. In this study, 35 taxonomical types and their isolates of *Pseudocercospora* species, mainly from Japan and Taiwan, were examined. In addition, the phylogenetic relationships among the species in this area were determined. These results contribute to a better understanding of fungal biodiversity and could help to prevent pandemics of important *Pseudocercospora* diseases in this area. Additional studies using authentic materials from Asian countries should be performed to reveal the fungal biodiversity of plant-parasitic fungi in this area, with a consideration of the biodiversity of the host plants involved.

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