
New species and newly recorded species of *Cercospora* and allied genera from Indonesia

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During a survey of plant parasitic fungi in Indonesia, we collected some species of morphologically unique fungi from leaf spots of herbal and arboreal plants. Eleven species belonging to the genus *Cercospora* and allied genera are newly added to the Indonesian mycoflora. Of these, two new *Pseudocercospora* species are described, *P. clerodendri-hastati* on *Clerodendrum hastatum* and *P. rhododendrigena* on *Rhododendron sinense*. A record of *Cercospora kyotensis* on *Dichroa febrifuga* is only the second since the species was observed on *Hydrangea* in Japan.

Key words – cercosporoid fungi – Indonesian mycoflora – *Pseudocercospora clerodendri-hastati* – *Pseudocercospora rhododendrigena* – rDNA sequences

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Introduction

The genus *Cercospora* was established by Fresenius in 1863 (Fuckel 1863, Braun 1995). The number of reported species belonging to this genus increases every year because most of these species are plant pathogenic and exhibit high host-specificity. In recent years, the genus *Cercospora* has been split into several genera on the basis of new criteria such as conidiomatal structure, and morphological features of the mycelia, conidiophores, conidiogenous cells, and conidial pigmentation (Crous & Braun 2003). There are now over 5500 species of cercosporoid fungi. The criteria used to distinguish the genera have been accepted by most investigators. However,

phylogenetic studies have shown that these criteria are partly unreliable at the generic level, subsequently leading to a reduction in the number of genera (Crous et al. 2000, 2001a,b, 2004a, 2006a,b, 2009a,b, Schubert & Braun 2005, Ayala-Escobar et al. 2006). However, the morphology and relationship between the species of cercosporoid fungi and their host plants remain major factors in taxonomic description. Most *Pseudocercospora* species are highly host-specific with a host range confined to a single genus or allied genera of a single family. However, molecular studies (Crous et al. 2001b, 2006a,b, Taylor et al. 2003, Periera & Barreto 2005, Den Breeÿen et al. 2006, Hunter et al. 2006) have not yet proved the host-specificity

of the fungi (Crous & Braun 2003, Crous et al. 2004b). Therefore, we tentatively applied the existing species concept of *Cercospora* and its allied genera, i.e., the species epithet is conferred on the basis of the distinguishable morphology on the leaves of a host genus or a host family.

The study of cercosporoid taxa in Indonesia was started by Boedijn (1961) who reported 90 species of *Cercospora* from 109 host plant species. However, these species were described on the basis of morphological characteristics provided by the genus concept of Chupp (1954). Using modern genus criteria, Braun (2001) recently re-examined Indonesian isotypes that were conserved at the CABI Bioscience UK Centre (IMI). Boedijn (1961) pointed out that the increase in number of the *Cercospora* species was due to the presence of a wide variety of vascular plants in Indonesia. However, the study of Indonesian species of *Cercospora* and its allied genera has not progressed despite their seemingly rich diversity. In this study, we describe cercosporoid fungi, including new species, after carrying out the repeated exploration of fungi in Indonesia.

Methods

The specimens were collected from the Cibodas Botanical Garden (6°44'00"S, 107°0'00"E) in Java and Eka Karya Botanical Garden (8°16'00"S, 115°9'00"E) in Bali in Indonesia. Slides for microscopic examination were prepared by hand sections from the freshly collected materials. Specimens were mounted in Shear's medium. Living cultures of single conidial isolates were obtained according to the protocol of Nakashima et al. (2011). Dried specimens were deposited in the Herbarium Bogoriense (BO) of the Research Center for Biogogy, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia. The cultures are maintained at Biotechnology Culture Collection (BTCC) in LIPI and at the National Institute of Technology and Evaluation (NBRC), Chiba, Japan. The sequence data of rDNA internal transcribed spacer (ITS) region of *Pseudocercospora* were deposited as the reference data for new species in the DNA data bank of Japan (DDBJ).

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing were

conducted under the conditions described by Kurihara et al. (2008) and Sukarno et al. (2009). Briefly, total DNA was extracted from an agar disc containing mycelium using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). ITS-1 and ITS-2 regions that included 5.8S rDNA and the D1 and D2 domains of large ribosomal subunit (LSU of rDNA), were amplified using KOD-Plus-DNA polymerase (Toyobo, Osaka, Japan) and the primers ITS-5 and NL4 (White et al. 1990, O'Donnell 1993) in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the conditions described by Kurihara et al. (2008). The primer NS7 (White et al. 1990) was used only occasionally instead of ITS-5, when needed. The products were purified with a QIAquick PCR Purification Kit (Qiagen). For sequencing of the ITS regions that included the 5.8S rDNA and the D1/D2 domains of LSU rDNA in both directions, the primers ITS-5, ITS-3, NL1, ITS-2, ITS-4, and NL4 (White et al. 1990, O'Donnell 1993) were used. Sequencing reactions were conducted using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) with a Biometra T-Gradient Cycler (Biometra, Göttingen, Germany) under the conditions described by Kurihara et al. (2008). The products were purified with CleanSEQ (Agencourt Bioscience, Beverly, MA, USA). Sequences were analyzed with a 3730xl Genetic Analyzer (Applied Biosystems). The sequences obtained in this study were assembled on 'ATGC' (Windows Ver.) Ver. 4.0.9 (GENETYX Co., Tokyo, Japan). Basic local alignment search tool (BLAST) search was performed to find the possible sister species of the newly sequenced taxa on the NCBI website.

A data set comprising the sequencing data from LSU rDNA including D1/D2 regions was indicated for revealing the taxonomic position of *Mycosphaerella* synanamorphs by Crous et al. (2009a). Thereupon, as a preliminary study, the taxonomic position of the isolates of the cercosporoid fungi obtained in this study were confirmed to cluster with each of the synanamorphs described by Crous et al. (2009a). For this study, we used the matrix of D1/D2 domains of 28S rDNA sequences downloaded from TreeBASE (accession number M4375; Crous et al. 2009a). The sequence data

of 326 taxa, consisting of 316 taxa referred from the downloaded alignments, and 9 *Pseudocercospora* isolates and an isolate of *Cercospora* s.l. which were obtained in this study, were aligned with ClustalW (Thompson et al. 1994) included in the MEGA4 (Tamura et al. 2007). Introns were deleted from the alignments, and ambiguously aligned positions were excluded from the data sets before performing analyses. The alignment gaps were treated as missing data. The phylogenetic trees were constructed by the maximum parsimony method using the heuristic search (CNI level = 3) option in MEGA4. Bootstrap (BS) values were subsequently calculated along with 100 replications to ensure reliability of the tree topologies. We found that Indonesian isolates could be classified into two genera namely, “Clade 16 *Pseudocercospora*” and “Clade 11 *Cercospora*,” which were described by Crous et al. (2009a). The BS values were 64 on the clade of *Pseudocercospora* and 88 on the clade of *Cercospora* (trees not shown).

Taxonomy

New species of the genus *Pseudocercospora*

Pseudocercospora clerodendri-hastati C. nakash., **sp. nov.** Figs 1, 4a–b
MycoBank 518794

Etymology – *clerodemdri-hastati*, derived from the name of the host plant.

Maculae folii vivi castaneae, angulares vel irregulares, 5–10 mm, hyphas internas et externas praeditae. Stromata amphigena, substomatata vel intraepidermalia, atro-brunnea, 24–45 μm diam. Conidiophora ex cellulis stromatis emergentia vel ex hyphis superficialibus oriunda, laxe vel dense fasciculata, brunnea, simplicia, recta vel geniculata, laevia, 10–37 \times 1.5–3 μm ; cellulae conidiogenae continuae, terminales, cum proliferationibus sympodialibus; loci conidiogeni inconspicui, non incrassati, non pigmentiferi. Conidia solitaria, acicularia vel obclavata, recta vel leniter curvata, laevia, pallide olivaceis, basi truncata, hila non incrassata, apice obtusa, 28–71 \times 1–2.5 μm , 3–9-septata.

Leaf spots distinct, reddish brown, angular to irregular, 5–10 mm in size. Caespituli amphigenous. Stromata substomatal to

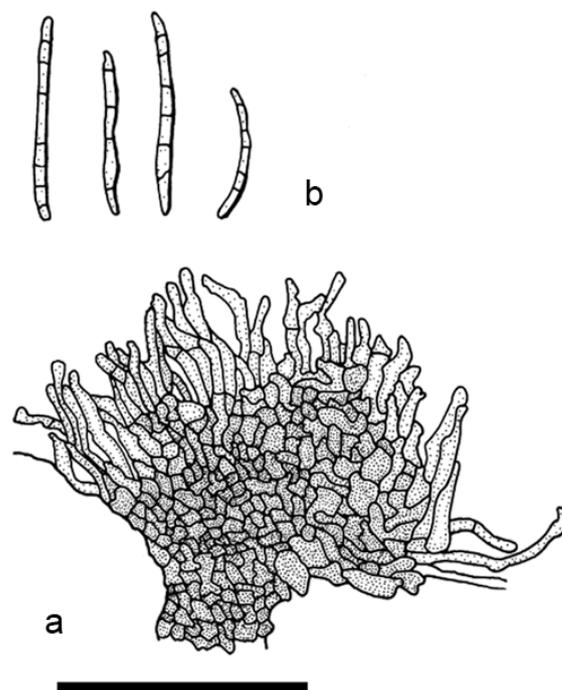


Fig. 1 – *Pseudocercospora clerodendri-hastati*. **a** Stromata and conidiophores. **b** Conidia. – Bar 50 μm

intraepidermal, distinct, small to well developed, dark brown, 14–67 μm in diameter, hyphae internal and external. Conidiophores arising from the upper part of stromata as well as from superficial hyphae on the under leaf surface, loosely to densely fasciculate, brown, simple, straight or well geniculate, smooth, 10–37 \times 1.5–3 μm . Conidiogenous cells integrated, terminal, proliferating sympodially; conidiogenous loci inconspicuous, unthickened, not darkened. Conidia solitary, acicular to obclavate, straight or slightly curved, smooth, pale brown to pale olivaceous, with unthickened and truncate basal end, apex obtuse, 28–71 \times 1–2.5 μm , 3–9-septate.

Material examined – Indonesia, Bali, Eka Karya Botanical Garden, on leaves of *Clerodendrum hastatum* Lindl. (Lamiaceae), 17 June 2003, C. Nakashima (BO22546, holotype) – ex-type cultures: NBRC105401 and BTCC F-61, deposited sequence of rDNA ITS1-5.8s-ITS2: AB453228.

Notes – Three species of *Pseudocercospora* are hitherto known on *Clerodendrum*: *P. clerodendri* (I. Miyake) Deighton (1976), *P. kashotoensis* (W. Yamam.) Deighton (1976), and *P. clerodendrigena* U. Braun (2002). *P. clerodendri-hastati* is morphologically similar

to *P. clerodendrigena* in terms of the size of well developed stromata, conidiophores, and conidia, but is different in that the former has 3–9-septate conidia (whereas the latter has 0–2-septate conidia) and superficial hyphae with solitary conidiophores. The other two species on *Clerodendrum*, i.e., *P. clerodendri* and *P. kashotoensis*, differ from *P. clerodendri-hastati* in that they have longer and wider conidia and poorly developed stromata. A *Mycosphaerella* species was observed on same plant specimen but any possible teleomorph-anamorph relationship between these fungi was not investigated. Further detailed examination is required to reveal their relationship.

A BLAST search with the ITS sequence of the present species (AB453228, 453bp) revealed several sequences of *Mycosphaerella* and its anamorphic fungi with a homology of over 98%. However, none completely matched the ITS sequences of the present species. The related fungal species were *Mycosphaerella cruenta* (GU214673, on *Vigna* sp., Fabaceae), *Pseudocercospora assamensis* (EU514281, on *Musa* sp., Musaceae), *P. atromarginalis* (GU214671, on *Solanum nigrum*, Solanaceae), *P. chengtuenensis* (GU214672, on *Lycium chinense*, Solanaceae), *P. fuligena* (GU214675, GU060636, on *Solanum lycopersicum*, Solanaceae), *P. lythracearum* (EF535720, on *Lagerstroemia indica*, Lythraceae), *P. tereticornis* (GQ852768, GQ852771, GQ852770, GQ852769, on *Eucalyptus* spp., Myrtaceae), and *Pseudocercospora* sp. (DQ676532, on *Chromolaena odorata*, Asteraceae). The sequence of cercosporoid fungi on *Clerodendrum*/Lamiaceae was not detected in any closely related species.

Pseudocercospora rhododendrigena C. Nakash., **sp. nov.** Figs 2, 4c,d
MycoBank 518795

Etymology – *rhododendrigena*, derived from the genus name of the host plant.

Maculae folii vivi disseminatae, angulares vel irregulares, 2–5 mm latitudo, atro-brunneae, Caespituli amphigeni. Hyphae internae et externae. Stromata nulla vel paucilocularia, raro evoluta, epiphylla, substromatalia vel intraepidermalia, brunnea vel atro-brunnea, 16–39 µm diam. Conidiophora solitaria, laxe vel dense fasciculata, ex cellulis stromatis emergentia vel ex hyphis superficialibus oriunda, pallide colorata vel pallide brunnea,

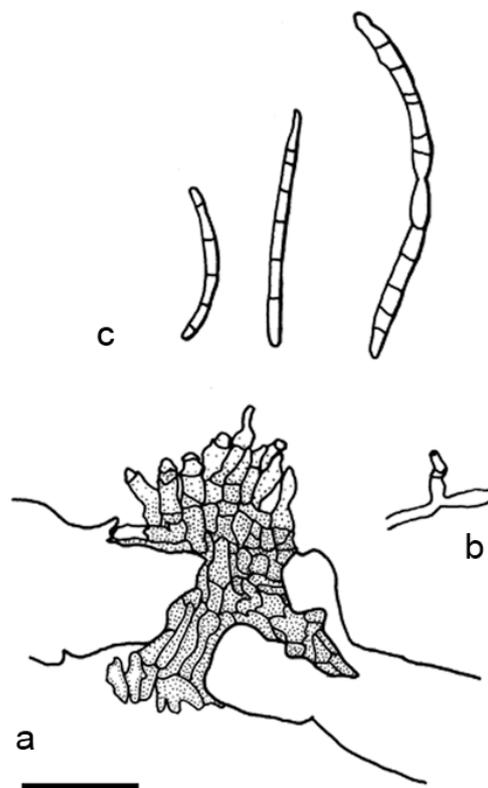


Fig. 2 – *Pseudocercospora rhododendrigena*. **a** Stromata and conidiophores. **b** Conidiophore emerging from external hyphae. **c** Conidia. – Bar 20 µm

simplicia, recta vel leviter geniculata, 2–24 × 2–3.5 µm. Cellulae conidiogenae continuae, terminales, cum proliferationibus percurrentibus vel sympodialibus. Loci conidiogeni inconspicui, non incrassati, non pigmentiferi. Conidia solitaria, filiformia vel obclavata, recta vel leviter curvata, laevia, pallide colorata, basi truncata, hila non incrassata, apice obtusa, 4–12-septata, constricta, 28–90 × 2–4 µm.

Leaf spots scattered, angular to irregular, dark brown, 2–5 mm wide. Caespituli amphigenous. Hyphae internal and external. Stromata lacking or composed of 2–3 brown cells, occasionally large stromata on upper leaf surface, substromatal to intraepidermal, brown to dark brown, 16–39 µm in diameter. Conidiophores emerging from stromata as well as external hyphae, pale coloured to pale brown, solitary or densely fasciculate, simple, straight to mildly geniculate, 2–24 × 2–3.5 µm, 1–3-septate. Conidiogenous cells integrated, terminal, proliferating sympodially or percurrently; conidiogenous loci unthickened, inconspicuous,

not darkened, not refractive. Conidia solitary, filiform to obclavate, straight to slightly curved, smooth, pale coloured, with unthickened hila, and obconically truncate basal end, apex obtuse, constricted at the septa, $28\text{--}90 \times 2\text{--}4 \mu\text{m}$, 4–12-septate.

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Rhododendron sinense* Sweet (Ericaceae), 23 July 2003, C. Nakashima (BO22547, holotype) – ex-type cultures: NBRC105400 and BTCC F-62, deposited sequence of rDNA ITS1-5.8s-ITS2: AB453227).

Notes – Two *Pseudocercospora* species are hitherto known on *Rhododendron*: *P. handelii* (Bubák) Deighton (1987) and *P. rhododendricola* (J.M. Yen) Deighton (1987). During the survey in Indonesia, two *Pseudocercospora* species were collected at one sampling site from two different species of *Rhododendron*. Of these, the fungus on *Rhododendron mucronatum* G. Don (BO22561) was identified as *P. rhododendricola* on the basis of its morphological characteristics. This fungus differs from *P. handelii* in that it has epiphyllous caespituli, denticulate and 1–2-septate conidiophores, and filiform and somewhat shorter and narrower ($54\text{--}96 \times 2\text{--}2.5 \mu\text{m}$) conidia (Shin & Kim 2001). However, the fungus on *R. sinense* collected in this study was not similar in morphological characteristics to the species known until now. Moreover, this new species causes severe leaf spot on the host without requiring an association with another organism, i.e., it is neither a saprobe nor a hyperparasite. Thus, we describe *P. rhododendrigena* as a new species, characterized by amphigenous caespituli, having various sizes of stromata and conidial shapes (constricted at the septa), and well developed external hyphae on the lower surface of the leaf spot.

The BLAST search results with the ITS sequence of *P. rhododendrigena* (AB453227, 452bp) showed that it matched the sequence of *P. elaeodendri* (GU980950, on *Tripterygium wilfordii*, Celastraceae), *P. indonesiana* (EU514283, on *Musa* sp., Musaceae), *Pseudocercospora* sp. (DQ184477, on *Syringa* sp., Oleaceae; DQ303082, on *Eucalyptus pellita*, Myrtaceae; DQ632694, on *Eucalyptus* sp., Myrtaceae; EF535712, on *Aleurites fordii*, Euphorbiaceae; and FJ425192, on *Malus* ×

domestica, Rosaceae). However, species belonging to the genus *Mycosphaerella* and its anamorphs on *Rhododendron*/Ericaceae were not identified as closely related.

Cercosporoid species new to the Indonesian mycoflora

Cercospora kyotensis M. Yoshik. & T. Yokoy., Trans. Mycol. Soc. Jpn. 33: 179, 1992. Fig 3

Known distribution – Japan (Yoshikawa & Yokoyama 1992).

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Dichroa febrifuga* Lour. (Hydrangeaceae), 23 July 2003, C. Nakashima (BO22562).

Notes – We were unable to obtain a culture of this fungus.

No cercosporoid species has been reported on the genus *Dichroa*. However, *C. kyotensis* characterized by protruding loci above the level of the conidiogenous cell wall has been reported from a Japanese specimen on *Hydrangea serrata* var. *thunbergii* (Siebold) H.

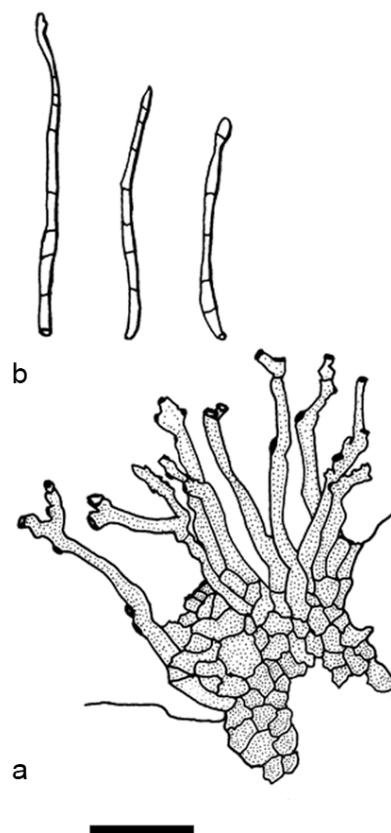


Fig 3 – *Cercospora kyotensis*. **a** Stromata and conidiophores. **b** Conidia. – Bar 20 μm

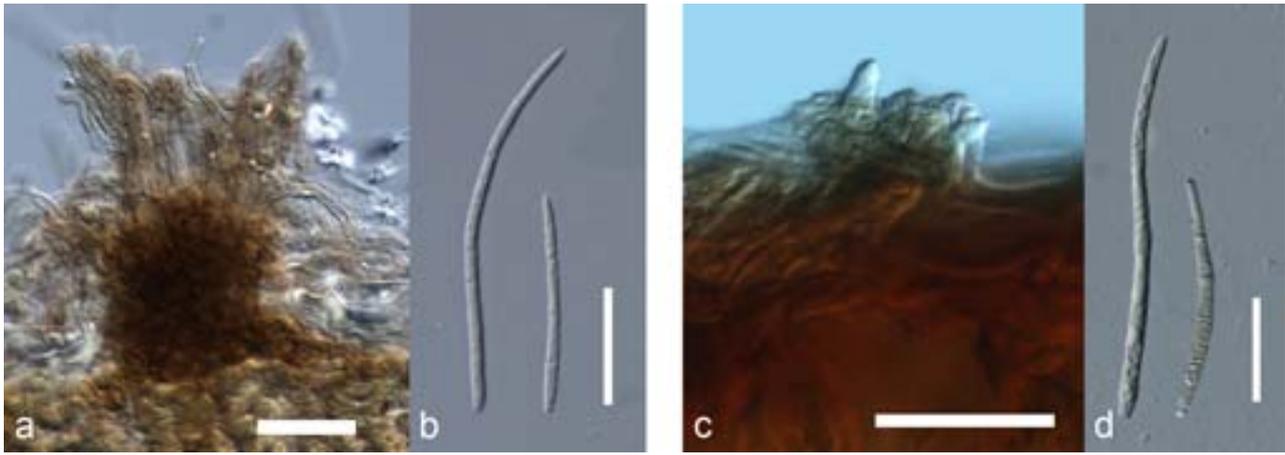


Fig 4 – **a** Vertical section of stroma and conidophores of *Pseudocercospora clerodendri-hastati*. **b** Conidia of *P. clerodendri-hastati*. **c** Vertical section of stroma and conidophores of *P. rhododendrigena*. **d** Conidia of *P. rhododendrigena*. – Bars = 20 μ m

Ohba (Hydrangeaceae). The fungus collected in the present study is morphologically similar to *C. kyotensis*. *Dichroa* is a new host of *C. kyotensis*.

Pseudocercospora acaciae-confusae (Sawada) Goh & W.H. Hsieh, Trans. Mycol. Soc. R.O.C. 4: 41, 1989.

Known distribution – Hong Kong, Libya, mainland China, Formosa (Crous & Braun 2003).

Material examined – Indonesia, Bali, Eka Karya Botanical Garden, on leaves of *Acacia auriculiformis* A. Cunn. ex Benth. (Leguminosae), 17 June 2003, C. Nakashima (BO22554) – cultures deposited: NBRC105403 and BTCC F-59.

Pseudocercospora asystasiae (J.M. Yen) J.M. Yen, Gard. Bull. Singapore 33: 169, 1980.

Known distribution – Brazil (Crous & Braun 2003).

Material examined – Indonesia, Bali, Eka Karya Botanical Garden, on leaves of *Asystasia nemorum* Nees (Acanthaceae), 17 June 2003, C. Nakashima (BO22549).

Notes – On the same specimen, *Cercospora asystasiana* J.M. Yen (cultures deposited as NBRC105395 and BTCC F-56) was observed.

Pseudocercospora buddlejae (W. Yamam.) Goh & W.H. Hsieh, Trans. Mycol. Soc. R.O.C. 2: 114, 1987.

Known distribution – China, India, Japan, Philippines, Taiwan, Thailand (Crous & Braun 2003, Nakashima et al. 2011).

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Buddleja asiatica* Lour. (Buddlejaceae), 19 June 2003, C. Nakashima (BO22555) – cultures deposited: NBRC105402 and BTCC F-60.

Pseudocercospora iteae (Sawada & Katsuki) Goh & W.H. Hsieh, Trans Mycol. Soc. R.O.C. 2: 136, 1987.

Known distribution – mainland China, Formosa (Crous & Braun 2003).

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Itea* sp. (Escalloniaceae), 19 June 2003, C. Nakashima – cultures deposited: NBRC105396 and BTCC F-66.

Pseudocercospora nojimai (Togashi & Katsuki) Y.L. Guo & X.J. Liu, Mycosystema 5: 105, 1992.

Known distribution – India, Japan, Korea, mainland China, Myanmar (Crous & Braun 2003).

Material examined – Indonesia, Bali, Eka Karya Botanical Garden, on leaves of *Impatiens* sp. (Balsaminaceae), 17 June 2003, C. Nakashima (BO22551) – cultures deposited: NBRC105405 and BTCC F-57.

Pseudocercospora rhododendricola (J.M. Yen) Deighton, Trans. Brit. Mycol. Soc. 88: 390, 1987.

Distribution – Hong Kong, Singapore (Crous & Braun 2003)

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Rhododendron mucronatum* G. Don (Ericaceae), 23 July 2003, C. Nakashima (BO22561) – cultures deposited: NBRC105397 and BTCC F-65.

Note: See also *P. rhododendrigena*.

Pseudocercospora violamaculans (Fukui) Tak. Kobay. & C. Nakash., Mycoscience 43: 225, 2002.

Known distribution – Japan, USA (Crous & Braun 2003).

Material examined – Indonesia, Java, Cibodas Botanical Garden, on leaves of *Rhaphiolepis indica* var. *umbellata* (Thunb. ex Murray) H. Ohashi (Rosaceae), 23 July 2003, C. Nakashima (BO22558) – cultures deposited: NBRC105399 and BTCC F-63.

Pseudocercospora wellesiana (Chupp) X.J. Liu & Y.L. Guo, Acta Mycol. Sin. 12: 32, 1993.

Known distribution – Borneo, China, India, Malaysia, Philippines (Crous & Braun 2003).

Material examined – Indonesia, Bali, Eka Karya Botanical Garden, on leaves of *Averrhoa carambola* L. (Oxalidaceae), 17 June 2003, C. Nakashima (BO22552) – cultures deposited: NBRC105404 and BTCC F-58.

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