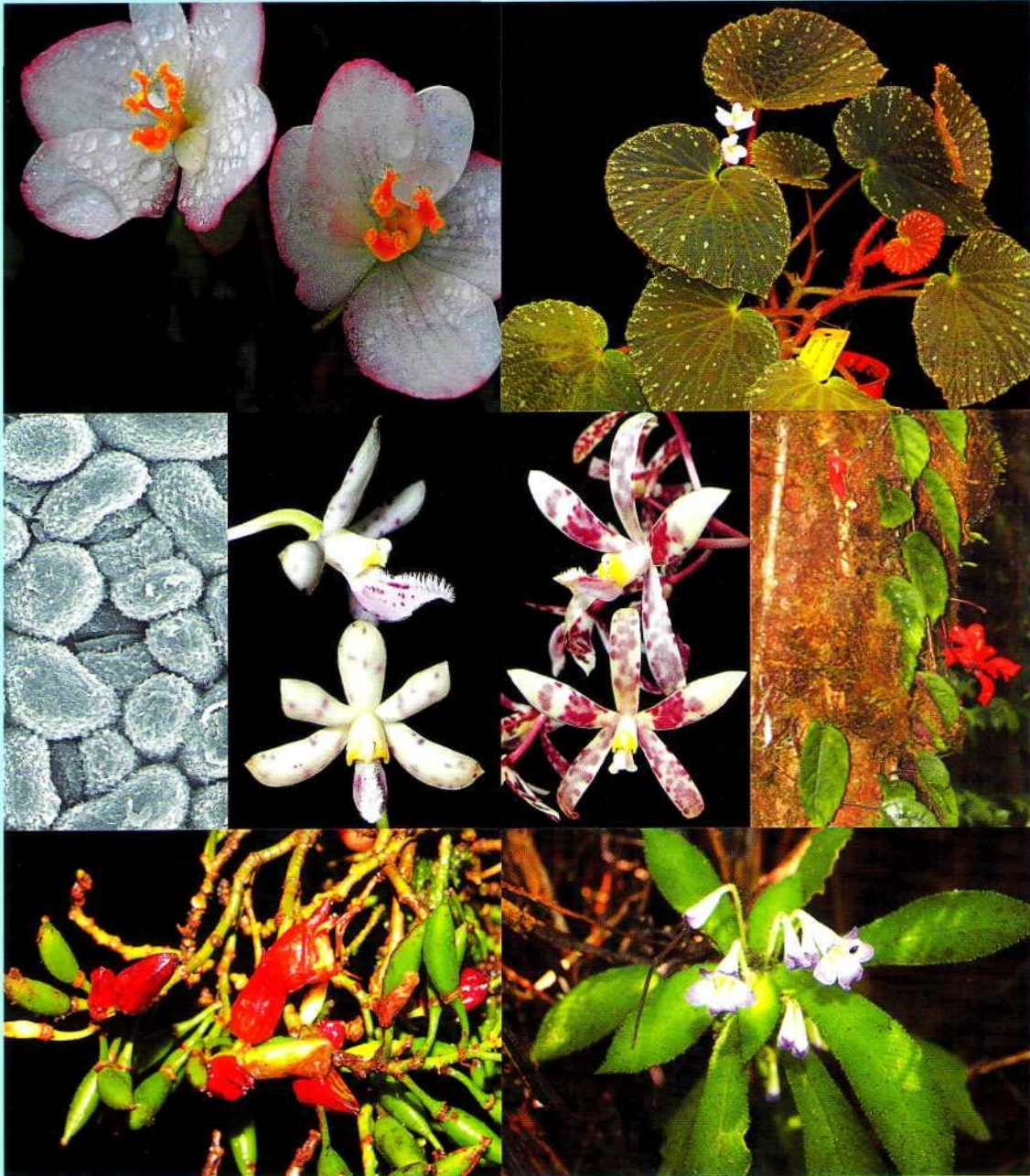




REINWARDTIA

A JOURNAL ON TAXONOMIC BOTANY, PLANT SOCIOLOGY AND ECOLOGY

ISSN 0034 – 365 X



2014 14 (1)

REINWARDTIA

A JOURNAL ON TAXONOMIC BOTANY,
PLANT SOCIOLOGY AND ECOLOGY

Vol. 14(1): 1-248, December 23, 2014

Chief Editor

Kartini Kramadibrata (Mycologist, Herbarium Bogoriense, Indonesia)

Editors

Dedy Darnaedi (Taxonomist, Herbarium Bogoriense, Indonesia)

Tukirin Partomihardjo (Ecologist, Herbarium Bogoriense, Indonesia)

Joeni Setijo Rahajoe (Ecologist, Herbarium Bogoriense, Indonesia)

Marlina Ardiyani (Taxonomist, Herbarium Bogoriense, Indonesia)

Topik Hidayat (Taxonomist, Indonesia University of Education, Indonesia)

Eizi Suzuki (Ecologist, Kagoshima University, Japan)

Jun Wen (Taxonomist, Smithsonian Natural History Museum, USA)

Managing Editor

Himmah Rustiami (Taxonomist, Herbarium Bogoriense, Indonesia)

Lulut Dwi Sulistyarningsih (Taxonomist, Herbarium Bogoriense, Indonesia)

Secretary

Endang Tri Utami

Layout Editor

Deden Sumirat Hidayat

Medi Sutiyatno

Illustrators

Subari

Wahyudi Santoso

Anne Kusumawaty

Correspondence on editorial matters and subscriptions for Reinwardtia should be addressed to:

HERBARIUM BOGORIENSE, BOTANY DIVISION,
RESEARCH CENTER FOR BIOLOGY- INDONESIAN INSTITUTE OF SCIENCES
CIBINONG SCIENCE CENTER, JLN. RAYA JAKARTA - BOGOR KM 46,
CIBINONG 16911, P.O. Box 25 Cibinong
INDONESIA
PHONE (+62) 21 8765066; Fax (+62) 21 8765062
E-MAIL: reinwardtia@mail.lipi.go.id

1	1
2	3 3 4
4	4

Cover images: 1. *Begonia holosericeoides* (female flower and habit) (Begoniaceae; Ardi *et al.*); 2. Abaxial cuticles of *Alseodaphne rhododendropsis* (Lauraceae; Nishida & van der Werff); 3. *Dipodium puspitae*, *Dipodium purpureum* (Orchidaceae; O'Byrne); 4. *Agalmyla exannulata*, *Cyrtandra coccinea* var. *celebica*, *Codonoboea kjellbergii* (Gesneriaceae; Kartonegoro & Potter).

The Editors would like to thanks all reviewers of volume 14(1):

- Abdulrohman Kartonegoro - Herbarium Bogoriense, Bogor, Indonesia
Altafhusain B. Nadaf - University of Pune, Pune, India
Amy Y. Rossman - Systematic Mycology & Microbiology Laboratory USDA-ARS, Beltsville, USA
Andre Schuiteman - Royal Botanic Gardens, Kew, UK
Ary P. Keim - Herbarium Bogoriense, Bogor, Indonesia
Barry Conn - Royal Botanic Gardens National Herbarium of New South Wales, Sydney, Australia
Dato' Abdul Latiff Mohamad - Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia
Daniel Potter - Department of Plant Sciences, University of California, Davis, California, USA
Deby Arifiani - Herbarium Bogoriense, Bogor, Indonesia
Ferry J. W. Slik - University of Brunei Darussalam, Brunei
Henti H. Rachmat - Conservation and Rehabilitation Research and Development Center, Bogor, Indonesia
Ian M. Turner - Royal Botanic Gardens, Kew, UK
Iskandar Z. Siregar - Bogor Agricultural University, Bogor, Indonesia
Jay H. Bernstein - Kingsborough Community College, Brooklyn, New York, USA
Jens G. Rohwer - University of Hamburg, Hamburg, Germany
Joan Pereira - SAN Herbarium, Sabah Forestry Department, Sabah, Malaysia
Kuswata Kartawinata - Herbarium Bogoriense, Bogor, Indonesia
Lars H. Schmidt - University of Copenhagen, Copenhagen, Denmark
Mark Hughes - Royal Botanic Gardens, Edinburgh, UK
Masahiro Kato - Kyoto University, Kyoto, Japan
Nuril Hidayati - Herbarium Bogoriense, Bogor, Indonesia
Ong Poh Teck - Forest Research Institute Malaysia, Kepong, Malaysia
Peter C. van Welzen - National Herbarium Netherlands, Leiden University Branch, Leiden, Netherlands
Reuben Nilus - Sabah Forestry Department, Sabah, Malaysia
Rugayah - Herbarium Bogoriense, Bogor, Indonesia
Ruth Kiew - Forest Research Institute of Malaysia, Kepong, Malaysia
Uwe Braun - Institut für Biologie Bereich Geobotanik und Botanischer Garten, Halle (Saale), Germany
Yasuaki Sato - Osaka-Sangyo University, Osaka, Japan

CERCOSPORA BRUNFELSIICOLA (FUNGI, MYCOSPHAERELLACEAE), A NEW TROPICAL CERCOSPOROID FUNGUS ON BRUNFELSIA UNIFLORA

Received January 17, 2014; accepted September 26, 2014

IMAN HIDAYAT

*Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia.
E-mail: imanhidayat@yahoo.com*

JAMJAN MEEBOON

Department of Agriculture, 50 Phaholyothin Rd., Ladyao, Chatuchack, Bangkok, Thailand.

ABSTRACT

HIDAYAT, I. & MEEBOON, J. 2014. *Cercospora brunfelsiicola* (fungi, Mycosphaerellaceae), a new tropical cercosporoid fungus on *Brunfelsia uniflora*. *Reinwardtia* 14(1): 211 – 217. — *Cercospora brunfelsiicola* on *Brunfelsia uniflora* is proposed as a new species based on a combination of molecular phylogenetic and morphological data analyses. The molecular phylogenetic analysis based on combined multilocus analyses of the Internal Transcribed Spacer (ITS), part of the elongation factor 1- α gene (EF1- α), and part of the calmodulin (CAL) gene regions showed that *C. brunfelsiicola* is phylogenetically distinguishable from other *Cercospora* species, including members of the *C. apii s. l.* complex. Morphologically, *C. brunfelsiicola* differs from other closely related *Cercospora* species, in particular *C. acaciae-mangii*, by forming lesions with indistinct margin, larger stromata [(32) 48.5 ± 10.6 (68) μm diam.], and filiform to narrowly obclavate conidia [(45) 59 ± 9.1 (72) \times (2.5) 2.5 ± 0.2 (3) μm].

Key words: Fungi, Hyphomycetes, leaf spot, phylogeny, taxonomy.

ABSTRAK

HIDAYAT, I. & MEEBOON, J. 2014. *Cercospora brunfelsiicola* (jamur, Mycosphaerellaceae), jenis jamur cercosporoid daerah tropis pada *Brunfelsia uniflora*. *Reinwardtia* 14(1): 211 – 217. — *Cercospora brunfelsiicola* Hidayat & Meeboon yang ditemukan pada bercak daun *Brunfelsia uniflora* diusulkan sebagai spesies baru berdasarkan kombinasi hasil analisis data morfologi dan analisis filogenetik sekuen DNA gabungan dari daerah *Internal Transcribed Spacer* (ITS), sebagian daerah gen *elongation factor 1- α gene* (EF1- α) dan gen *calmodulin* (CAL). Hasil analisis menunjukkan bahwa *C. brunfelsiicola* secara filogenetik terpisah dari sekuen DNA *Cercospora* terdekat, termasuk anggota *C. apii s.l.* Secara morfologi, *C. brunfelsiicola* dapat dibedakan dari spesies *Cercospora* terdekat, khususnya *C. acaciae-mangii*, karena memiliki warna gejala pada daun kecoklatan dengan garis batas yang tidak jelas, stromata lebih besar [diameter (32) $48,5 \pm 10,6$ (68) μm] dan memiliki bentuk konidia *obclavate* [(45) $59 \pm 9,1$ (72) \times (2,5) $2,5 \pm 0,2$ (3) m].

Kata kunci: Bercak daun, filogeni, Hifomisetes, jamur, taksonomi.

INTRODUCTION

Cercospora Fresen. is an anamorph-typified ascomycete genus, now considered and used as holomorph genus covering species only forming *Cercospora*-like asexual morphs as well as species with such asexual and *Mycosphaerella*-like sexual morphs (Braun *et al.*, 2013). More than 3000 names have been described worldwide (Crous *et al.*, 2000; Crous & Braun, 2003) [see also <http://www.mycobank.com/Mycotaxo.aspx>]. Members of this genus are commonly found as saprobes, secondary invaders and plant pathogens causing leaf spot or leaf blight (Crous & Braun, 2003). The morphological characteristics of pigmented conidiophores, conspicuously thickened and darkened conidiogenous loci (scars) and conidial

hila and scolecosporous, mostly acicular, pluriseptate, hyaline conidia, are typical for the genus *Cercospora* (Crous & Braun, 2003). Recent studies of cercosporoid fungi from Thailand have revealed several new species and new records of *Cercospora* on wild plants and economically important cultivated plants (Meeboon *et al.*, 2007a, b, c; Nakashima *et al.*, 2010; To-anun *et al.*, 2009, 2011; Phengsintham *et al.*, 2012, 2013).

During the study of diversity of *Cercospora* and allied genera in Thailand (2008–2010), we found *Cercospora* leaf spot on an ornamental plant identified as *Brunfelsia uniflora* [syn. *B. hopeana*] at Royal Flora Garden, Chiang Mai. This plant belongs to family Solanaceae, originally from South America and now widely cultivated as ornamental plant. *Brunfelsia uniflora* is also recognized as

medicinal plants for diuretic, antirheumatic and anti-inflammatory (Plowman, 1977). In this report, *Cercospora* specimen on *B. uniflora* from Thailand is proposed as a new species based on a combination of morphology and molecular phylogenetic analyses involving ITS rDNA, part of the elongation factor 1- α (EF1- α) gene and part of the calmodulin (CAL) gene region.

MATERIALS AND METHODS

Collection and Observation

Specimens of *Cercospora* leaf spot on *B. uniflora* were collected from Royal Flora, Chiang Mai Province, Thailand. Magnifying lenses of 10 \times and 20 \times magnifications were used during the

observation of symptoms in the field. Specimens showing the presence of *Cercospora caespituli* were placed in plastic bags for further examination. The collecting bags were sealed and labeled as follows: name of host plant, collection site, collector/s and collection date.

Macroscopic characteristics were observed using a stereo microscope (Olympus SZX7) to check the fungal caespituli on the leaf spots in detail. The examination of microscopic characters was carried out by means of an Olympus BX53 light microscope using oil immersion (1000 \times). Specimens for observation were prepared by hand sectioning. Water and Shear's solution were used as mounting media. Thirty conidia, hila, conidiophores, conidiogenous loci and 10 stromata

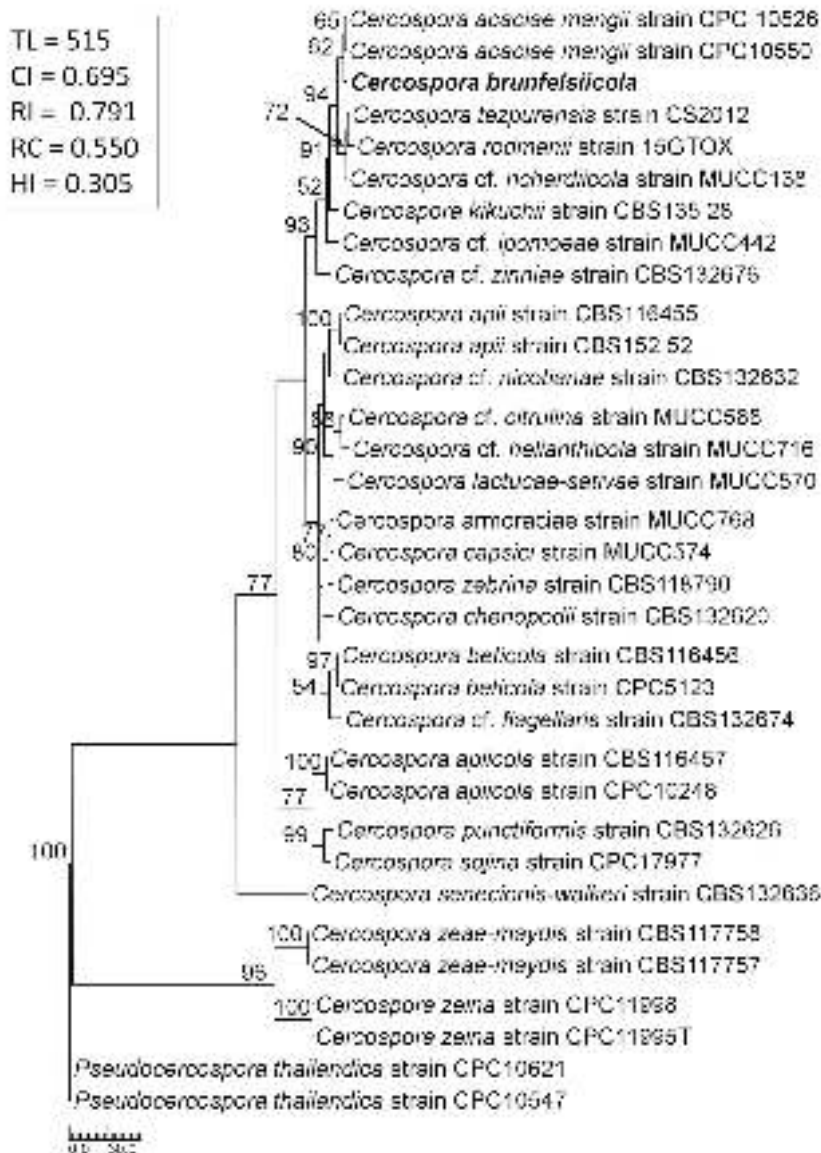


Fig. 1. Phylogenetic tree based on combination of ITS, partial EF1- α , and CAL genes region representing placement of members of *C. brunfelsicola* within *Cercospora s.str.*. Bootstrap support $\geq 50\%$ from Maximum Parsimony analysis are shown on the nodes.

were measured for each specimen. Line drawings were prepared at a magnification of 400× or 1000×. Single spore isolation was carried out following the method outlined by Choi *et al.* (1999) with a modification. Dried herbarium specimens were deposited in the BIOTEC Herbarium (BBH), Thailand. Cultures were deposited at BIOTEC culture collection (BCC), Thailand and LIPI microbial culture collection (LIPIMC), Indonesia.

DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing

A culture of *Cercospora* isolated from *B. uniflora* was grown on potato dextrose broth (PDB, Difco) and incubated on waterbath shaker (100 rpm) (Taitec Personal-11) for 10 days at 28°C prior to DNA extraction. Fungal mycelia were harvested in a 1.5 ml tube with 500 µl miliQ water. Mycelial cell-walls were homogenized mechanically using

Table 1. GenBank accession number of cercosporoid fungi used in this study.

No.	Species	Strain Code	GenBank Accession Number		
			ITS	EF	CAL
1	<i>Cercospora brunfelsiicola</i>	RF5	AB859638	AB863025	AB863026
2	<i>Cercospora acaciae-mangii</i>	CPC 10526T	AY752141	AY752176	AY752235
3	<i>Cercospora acaciae-mangii</i>	CPC 10550	AY752139	AY752172	AY752231
4	<i>Cercospora senecionis-walkeri</i>	CBS 132636	JX143649	JX143408	JX142916
5	<i>Cercospora apii</i>	CBS 116455	AY840519	AY840486	AY840417
6	<i>Cercospora apiicola</i>	CBS 116457	AY840536	AY840503	AY840434
7	<i>Cercospora armoraciae</i>	MUCC 768	JX143554	JX143308	JX142816
8	<i>Cercospora apii</i>	CBS 152.52	AY840515	AY840482	AY840413
9	<i>Cercospora apiicola</i>	CPC 10248	AY840539	AY840506	AY840437
10	<i>Cercospora beticola</i>	CBS 116456	AY840527	AY840494	AY840425
11	<i>Cercospora cf. flagellaris</i>	CBS 132674	JX143606	JX143364	JX142872
12	<i>Cercospora capsici</i>	MUCC 574	JX143569	JX143325	JX142833
13	<i>Cercospora chenopodii</i>	CBS 132620	JX143571	JX143327	JX142835
14	<i>Cercospora cf. citrulina</i>	MUCC 588	JX143582	JX143340	JX142848
15	<i>Cercospora tezpurenensis</i>	CS 2012T	KC351743	KC513746	KC513745
16	<i>Cercospora zae-maydis</i>	CBS 117758	DQ185075	DQ185087	DQ185111
17	<i>Cercospora zeina</i>	CPC 11998	DQ185082	DQ185094	DQ185118
18	<i>Cercospora beticola</i>	CPC 5123	DQ233327	DQ185094	DQ233405
19	<i>Cercospora cf. helianthicola</i>	MUCC 716	JX143615	JX143374	JX142882
20	<i>Cercospora cf. ipomoeae</i>	MUCC 442	JX143618	JX143377	JX142885
21	<i>Cercospora kikuchii</i>	CBS 135.28	DQ835071	DQ835089	DQ835135
22	<i>Cercospora lactucae-sativae</i>	MUCC 570	JX143623	JX143382	JX142890
23	<i>Cercospora cf. nicotianae</i>	CBS 132632	JX143631	JX143390	JX142898
24	<i>Cercospora punctiformis</i>	CBS 132626	JX143638	JX143397	JX142905
25	<i>Cercospora cf. richardiicola</i>	MUCC 138	JX143643	JX143402	JX142910
26	<i>Cercospora rodmanii</i>	15-GTOX	GQ884185	GQ884191	GQ884195
27	<i>Cercospora sojina</i>	CPC 17977	JX143674	JX143434	JX142942
28	<i>Cercospora zae-maydis</i>	CBS 117757	DQ185074	DQ185086	DQ185110
29	<i>Cercospora zebrina</i>	CBS 118790	GU214657	KF253248	KF253963
30	<i>Cercospora zeina</i>	CPC 11995T	DQ185081	DQ185093	DQ185117
31	<i>Cercospora cf. zinniae</i>	CBS 132676	JX143757	JX143519	JX143027
32	<i>Pseudocercospora thailandica</i>	CPC 10621	AY752159	AY752189	AY752251
33	<i>Pseudocercospora thailandica</i>	CPC 10547	AY752156	DQ835102	AY752248

plastic pestle. The mycelia were then centrifuged using a Centrifuge MiniSpin (Eppendorf, Germany) at 14.500 rpm for 10 minutes. DNA was extracted from the mycelia with a PHYTOPURE™ DNA extraction kit (GE Healthcare, UK) according to the manufacturer's instruction. DNA amplification was performed by polymerase chain reaction (PCR) using primer pairs of ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for ITS (Internal Transcribed Spacer) region (White *et al.*, 1990), EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3') for part of Elongation Factor 1- α region, and CAL-228F (5'-GAGTTCAAGGAGGCCTTCTCCC-3') and CAL-737R (5'-CATCTTTCTGGCCATCATGG-3') for Calmodulin region (Carbone & Kohn, 1999). For the ITS region, PCR was performed in a 25 ml volume as follows: nuclease free water 10 μ l, Go Taq® Green Mastermix (Promega, Madison, USA) 12.5 μ l, ITS5 dan ITS4 primer 0.5 μ l for each primer, DMSO 0.5 μ l, and DNA template 1 μ l. The PCR reaction was done using TaKaRa thermocycler (TaKaRa, Japan) as follows: initial denaturation at 95°C for 90 s, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 90 s, and final extension of 72°C for 5 min. PCR reactions for EF and CAL genes were performed in 25 mL reaction volumes as follow: each reaction containing nuclease free water 8.75 μ l, Go Taq® Green Mastermix (Promega, Madison, USA) 12.5 μ l, forward and reverse primer 0.625 μ l for each primer, DMSO 0.5 μ l, and DNA template 2 μ l. PCR was performed in a TaKaRa thermocycler (TaKaRa, Japan) with the following program: 94°C for 5 min, 35 cycles {94°C for 30 s, 52°C for 30 s, 72°C for 30 s} and followed by a final extension of 7 min at 72°C (Groenewald *et al.*, 2005). PCR results were visualized using electrophoresis method in a 1% agarose gel at 100 V for 30 min. Agarose gel was soaked in an ethidium bromide for 60 mins and visualized under UV light (Printgraph). PCR products were sent to 1stBase (Malaysia) for DNA sequencing.

Phylogenetic Analysis

Nucleotide sequences obtained from the respective primer pairs (ITS5 and ITS4, EF1-728F and EF1-986R, CAL-228F and CAL-737R) were examined and refined by direct examination using Chromas Pro 1.41 software (Technelysium Pty Ltd., Australia). Sequences generated from the respective ITS, EF, and CAL regions were aligned with sequences retrieved from DNA databases (DDBJ, NCBI) using MUSCLE (Edgar, 2004) implemented

in MEGA 6 (Tamura *et al.*, 2013). *Pseudocercospora thailandica* strain CPC 10621 and *P. thailandica* strain CPC 10547 were used as outgroups in the analysis. Regions designated as ambiguously aligned were excluded from the analyses. GeneBank accession number, strain code, and taxon names used in this study are given in Table 1.

The phylogenetic analysis was conducted using the maximum parsimony (MP) method in PAUP* 4.0b10 (Swofford, 2002). The MP analysis was performed with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm with 1000 random sequence additions to find the optimum tree. The stepwise addition option set as random and maximum tree number was set at 5000. Tree length (TL), consistency index (CI), retention index (RI), related consistency index (RC), and homoplasy index (HI) were also calculated. The Kishino-Hasegawa (KH) likelihood test (Kishino & Hasegawa, 1989) was carried out to compare the best tree topology obtained by the nucleotide sequence data with a constrained tree. The strength of the internal branches of the phylogenetic tree in MP analysis was tested with bootstrap (BS) analysis (Felsenstein, 1985) using 1000 replications. BS values of 50 % or higher are shown. Random sequence addition was used in the bootstrap analysis. All sites were treated as unordered and unweighted, and gaps treated as missing data. TreeGraph 2 (Stöver & Müller, 2010) was used to refine the phylogenetic tree. The partition homogeneity test (Farris *et al.*, 1995) was carried out by using PAUP* to determine whether ITS, EF1- α , and CAL datasets were in conflict with 1000 replicates.

RESULTS

Phylogenetic Analysis

The alignment of the combined sequences from ITS, EF and CAL regions contained 33 sequences and 1120 total characters, of which 835 characters were constant, 40 characters were variable and parsimony-uninformative, 245 characters were parsimony-informative. The most parsimonious tree was generated in 515 steps (CI = 0.695, RI = 0.791, RC = 0.550, HI = 0.305). The phylogenetic tree generated from MP analysis showed that *C. brunfelsiicola* is phylogenetically distinguishable from other *Cercospora* sequences (Fig. 1). Sequences of *C. brunfelsiicola* formed an independent lineage and showed a close relationship to sequences of *C. acaciae-mangii* Crous, Pongpan. & M. J. Wingf. on *Acacia mangium* Willd. (Leguminosae) with 62% BS (bootstrap support).

This clade is sister to a clade containing sequences of *C. tezpurenensis* M. K. Meghvansi & Md. Haneef Khan on *Capsicum assamicum* Purkayastha & Singh, *C. rodmanii* on *Eichhornia crassipes* (Mart.) Solms, and *C. cf. richardiicola* on *Fuchsia*×*hybrida* with 94% BS.

Taxonomy

Cercospora brunfelsiicola Hidayat & Meeboon, *spec. nov.* – Fig. 2.

MYCOBANK MB805994

Cercospora brunfelsiicola differs from *C. acaciae-mangii* and other closely related *Cercospora* species by forming lesions with indistinct margin, larger stromata [(32) 48.5 ± 10.6 (68) μm diam.], and filiform to narrowly obclavate conidia [(45) 59 ± 9.1 (72) \times (2.5) 2.5 ± 0.2 (3) μm]. — Type: Thailand, Chiang Mai Province, Royal Flora

Garden, on leaves of *Brunfelsia uniflora* (Pohl) D. Don [= *B. hopeana* (Hook.) Benth.] (Solanaceae), 27 July 2008, Jamjan Meeboon, RF5 (BBH 23764: Holotype). Ex-type culture: BCC32756, other culture: LIPIMC 774. GenBank accession number (ITS: AB859638, EF: AB863025, CAL: AB863026).

Leaf spots amphigenous, distinct, circular to angular, 2–7 mm diam., brown, sometimes forming larger lesions, margin indistinct. *Caespituli* mainly epiphyllous, dark or blackish, punctiform, scattered within the lesions. *Stromata* (32) 48.5 ± 10.6 (68) μm diam. (n = 10), intraepidermal, well-developed, composed of globular to angular, brown to blackish brown cells. *Conidiophores* numerous, in dense fascicles arising from stromata, (34) 98.5 ± 28.8 (151) \times (2.5) 4 ± 0.6 (5.5) μm (n = 30), rarely branched, subcylindrical, strongly geniculate, 2–5-septate, simple, straight, erect to decumbent, smooth, pale

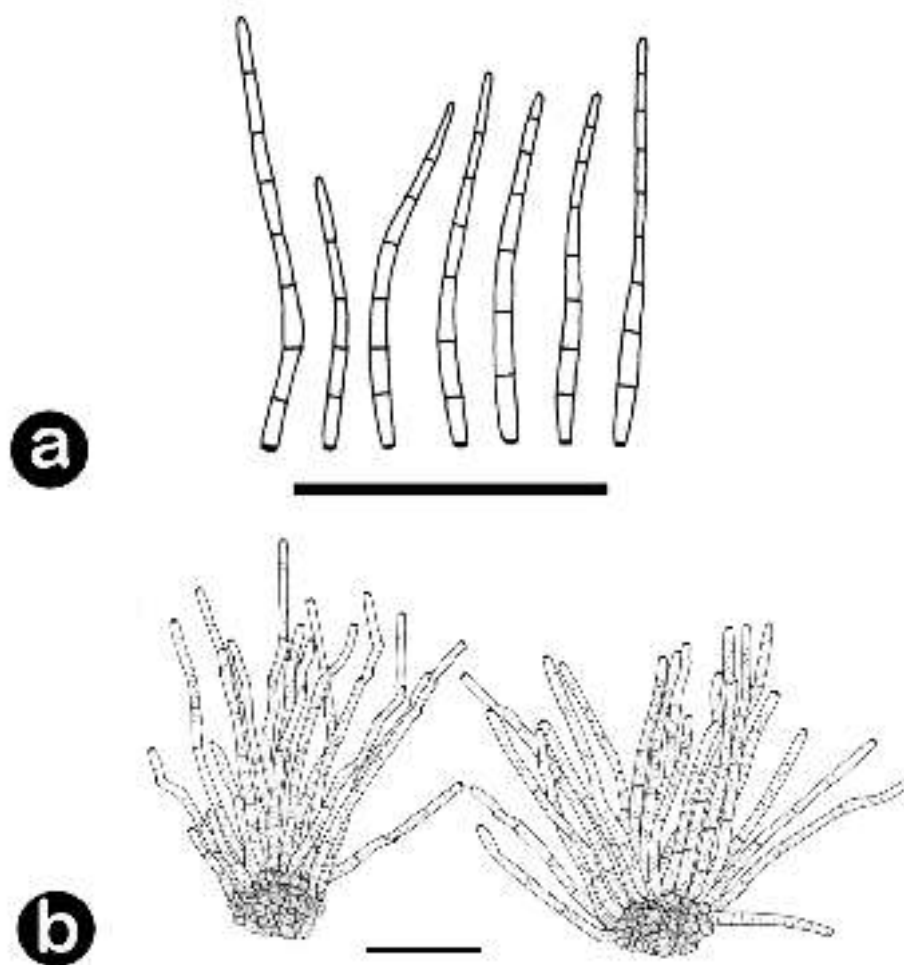


Fig. 2. *Cercospora brunfelsiicola* sp. nov. (from holotype) a. Conidia. b. Stromata and conidiophores. (Scale bar = 50 μm).

yellow to pale brown. *Conidiogenous cells* integrated, terminal, (10) 21.5 ± 8.2 (47.2) \times (2.5) 4.1 ± 1.1 (4.8) μm long (n = 30), holoblastic, monoblastic to polyblastic, sympodially proliferating. *Conidiogenous loci* 2.5–3 μm diam. (n = 30), conspicuous, thickened and darkened. *Conidia* (45) 59 ± 9.1 (72) \times (2.5) 2.5 ± 0.2 (3) μm (n = 30), solitary, filiform to narrowly obclavate, 4–8-septate, straight, hyaline, smooth, base obconically truncate, with subacute apex, hila 2–2.5 μm diam. (n = 30), thickened and darkened.

Distribution. Only known from its type locality.

Etymology. The new species is named after its host generic name.

DISCUSSION

The current study is the first report of *Cercospora* found on *B. uniflora*. About 12 species of *Cercospora* have been recognized on hosts of the plant family Solanaceae, viz. *C. canescens* Ellis & G. Martin (*C. apii* s. l.), *C. lanugiflori* Chupp & A.S. Mull. [on *Solanum velutinum* Dunal (= *S. lanugiflorum* Pittier)], *C. nigri* Tharp var. *microspora* L. N. Bhardwaj & Y. S. Paul (on *S. nigrum* L.), *C. nicandrae* Chupp [on *Nicandra physalodes* (L.) Gaertn.], *C. nicotianicola* J. M. Yen (on *Nicotiana tabacum* L.), *C. physalidis* Ellis (*C. apii* s. lat.), *C. physalidis-angulatae* J.M. Yen (on *Physalis angulata* L.), *C. puyana* Syd. (on *S. trachycyphum* Bitter), *C. sciadophila* (Speg.) Chupp (on *S. violifolium* Schott. ex Spreng), *C. solanacea* Sacc. & Berl. (on *Solanum* spp.), *C. solani* Thüm. (on *Solanum* spp.), and *C. Solani-nigri* Chidd. (on *S. nigrum*) [Chupp, 1954; Crous & Braun, 2003]. *Cercospora venezuelae* var. *indica* Govindu & Thirum. and *C. solanigena* Bhartiya, R. Dubey & S. K. Singh are noted as uncertain species by Crous & Braun (2003) as type material could not be traced and due to the assumption that the original description was based on young conidia, respectively. Based on the morphological examination, *C. brunfelsiicola* is distinguishable from other *Cercospora* species found on hosts of the plant family Solanaceae by having strongly geniculate, densely fasciculate conidiophores and filiform to narrowly obclavate conidia with a few septa (Fig. 2). *Cercospora brunfelsiicola* differs from the plurivorous *C. apii* s. l. on Solanaceae (*C. canescens*, *C. physalidis*) by having well-developed stromata, numerous conidiophores in dense fascicles and strongly geniculate conidiophores. The filiform to narrowly

obclavate conidia of *C. brunfelsiicola* (Fig. 2) are apparently distinct from the long acicular conidia of *C. apii* s. l.

The molecular phylogenetic analyses based on combined sequence of ITS, EF1- α and CAL gene regions apparently showed that *C. brunfelsiicola* has a close phylogenetic relationship to *C. acaciae-mangii* (Groenewald *et al.*, 2013). *Cercospora brunfelsiicola* is distinguishable from *C. acaciae-mangii* on *A. mangium*, which was originally described from Thailand as well (Crous *et al.*, 2004), by having lesions with indistinct margin, larger stromata [(32) 48.5 ± 10.6 (68) μm diam.] and filiform to narrowly obclavate conidia [(45) 59 ± 9.1 (72) \times (2.5) 2.5 ± 0.2 (3) μm]. *Cercospora acaciae-mangii* was morphologically described by Crous *et al.* (2004) based on distinct symptom (lesions medium brown, surrounded by a raised, dark brown border), stromata lacking to well-developed (up to 30 μm diam.) and conidia acicular (50–350 \times 3.5–5 μm). In conclusion, our morphological data showed that the lesions with indistinct margin, densely fasciculate, strongly geniculate conidiophores and filiform to narrowly obclavate conidia are distinct characters that distinguish *C. brunfelsiicola* from other closely related *Cercospora* species and justify the introduction of a new species for this fungus.

ACKNOWLEDGEMENTS

This research was financially supported by a research grant from the International Foundation for Science, awarded to Jamjan Meeboon (D/45381). The authors thank Ms. Floretta F. Yuliani for technical assistance during DNA extraction and PCR amplification.

REFERENCES

- BRAUN, U., NAKASHIMA, C. & CROUS, P. W. 2013. Cercosporoid fungi (*Mycosphaerellaceae*) 1. Species on other fungi, *Pteridophyta* and *Gymnospermae*. *IMA Fungus* 4: 265–345.
- CARBONE, I. & KOHN, L. M. 1999. A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia* 9: 553–556. <http://dx.doi.org/10.2307/3761358>.
- CHOI, Y. W., HYDE, K. D. & HO, W. H. 1999. Single spore isolation of fungi. *Fungal Diversity* 3: 29–38.
- CHUPP, C. 1954. *A monograph of the fungus genus Cercospora*. Ithaca, New York.
- CROUS, P. W. & BRAUN, U. 2003. *Mycosphaerella* and its anamorphs, 1. Names published in *Cercospora* and *Passalora*. *CBS Biodiversity Series* 1. Utrecht, Netherland. Pp. 571.
- CROUS, P. W., APTROOT, A., KANG, J. C., BRAUN, U. & WINGFIELD, M. J. 2000. The

- genus *Mycosphaerella* and its anamorphs. *Studies in Mycology* 45: 107–121.
- CROUS, P. W., GROENEWALD, J. Z., PONGPANICH, K., HIMAMAN, W., ARZANLOU, M. & WINGFIELD, M. J. 2004. Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. *Studies in Mycology* 50: 457–469.
- EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research* 32: 1792–1797. <http://dx.doi.org/10.1093/nar/gkh340>.
- FARRIS, J. S., KALLERSJÖ, M., KLUGE, A. G. & BULT, C. 1995. Constructing a significance test for incongruence. *Syst. Biol.* 44: 570–572.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- GROENEWALD, M., GROENEWALD, J. Z. & CROUS, P. W. 2005. Distinct species exist within the *Cercospora apii* morphotype. *Phytopathology* 95: 951–959.
- GROENEWALD, J. Z., NAKASHIMA, C., NISHIKAWA, J., SHIN, H. D., PARK, J. H., JAMA, A. N., GROENEWALD, M., BRAUN, U. & CROUS, P. W. 2013. Species concepts in *Cercospora*: spotting the weeds among the roses. *Studies in Mycology* 75: 115–170. <http://dx.doi.org/10.3114/sim0012>.
- KISHINO, H. & HASEGAWA, M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29: 170–179.
- MEEBOON, J., HIDAYAT, I., NAKASHIMA, C. & TO-ANUN, C. 2007a. *Cercospora habenariicola* sp. nov. and some new records of cercosporoid fungi from Thailand. *Mycotaxon* 99: 117–121.
- MEEBOON, J., HIDAYAT, I. & TO-ANUN, C. 2007b. Cercosporoid fungi from Thailand 3. Two new species of *Passalora* and six new records of *Cercospora* species. *Mycotaxon* 102: 139–145.
- MEEBOON, J., HIDAYAT, I. & TO-ANUN, C. 2007c. An annotated list of cercosporoid fungi in Northern Thailand. *Journal of Agricultural Technology* 3: 51–63.
- NAKASHIMA, C., OETARI, A., KANTI, A., SARASWATI, R., WIDYASTUTI, Y. & ANDO, K. 2010. New species and newly recorded species of *Cercospora* and allied genera from Indonesia. *Mycosphere* 1: 315–323.
- PHENGSIKHAM, P., CHUKEATIROTE, E., MCKENZIE, E. H. C., MOSLEM, M. A., HYDE, K. D. & BRAUN, U. 2012. Fourteen new records of cercosporoids from Thailand. *Maejo International Journal of Science and Technology* 6: 47–61.
- PHENGSIKHAM, P., BRAUN, U., MCKENZIE, E. H. C., CHUKEATIROTE, E., CAI, L. & HYDE, K. D. 2013. Monograph of cercosporoid fungi from Thailand. *Plant Pathology and Quarantine* 3: 67–138.
- PLOWMAN, T. 1977. *Brunfelsia* in ethnomedicine. *Botanical Museum leaflets* 25: 289–320.
- STÖVER, B. C. & MÜLLER, K. F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* 11: 7. <http://dx.doi.org/10.1186/1471-2105-11-7>.
- SWOFFORD, D. L. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4*. Sinauer Associates, Sunderland, Massachusetts.
- TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013 – MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. <http://dx.doi.org/10.1093/molbev/mst197>.
- TO-ANUN, C., HIDAYAT, I. & MEEBOON, J. 2011. Genus *Cercospora* in Thailand: Taxonomy and phylogeny (with a dichotomous key to species). *Plant Pathology & Quarantine* 1: 11–87.
- WHITE, T. J., BRUNS, T., LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322 pp. In: INNIS, M. A., *et al.* (Eds.), *PCR Protocols: a guide to methods and applications*. San Diego, Academic Press.

INSTRUCTION TO AUTHORS

Scope. *Reinwardtia* is a scientific irregular journal on plant taxonomy, plant ecology and ethnobotany published in December. Manuscript intended for a publication should be written in English.

Titles. Titles should be brief, informative and followed by author's name and mailing address in one-paragraphed.

Abstract. English abstract followed by Indonesian abstract of not more than 250 words. Keywords should be given below each abstract.

Manuscript. Manuscript is original paper and represent an article which has not been published in any other journal or proceedings. The manuscript of no more than 200 pages by using Times New Roman 11, MS Word for Windows of A4 with double spacing, submitted to the editor through <reinwardtia@mail.lipi.go.id>. New paragraph should be indented in by 5 characters. For the style of presentation, authors should follow the latest issue of *Reinwardtia* very closely. Author(s) should send the preferred running title of the article submitted. Every manuscript will be sent to two blind reviewers.

Identification key. Taxonomic identification key should be prepared using the aligned couplet type.

Nomenclature. Strict adherence to the International Code of Botanical Nomenclature is observed, so that taxonomic and nomenclatural novelties should be clearly shown. English description for new taxon proposed should be provided and the herbaria where the type specimens area deposited should be presented. Name of taxon in taxonomic treatment should be presented in the long form that is name of taxon, author's name, year of publication, abbreviated journal or book title, volume, number and page.

Map/line drawing illustration/photograph. Map, line drawing illustration, or photograph preferably should be prepared in landscape presentation to occupy two columns. Illustration must be submitted as original art accompanying, but separated from the manuscript. The illustration should be saved in JPG or GIF format at least 350 pixels. Legends or illustration must be submitted separately at the end of the manuscript.

References. Bibliography, list of literature cited or references follow the Harvard system as the following examples.

- Journal : KRAENZLIN, F. 1913. *Cyrtandraceae* novae Philippinenses I. *Philipp. J. Sci.* 8: 163-179.
MAYER, V., MOLLER, ML, PERRET, M. & WEBER, A. 2003. Phylogenetic position and generic differentiation of *Epithemateae* (*Gesneriaceae*) inferred from plastid DNA sequence data. *American J. Bot.* 90: 321-329.
- Proceedings : TEMU, S. T. 1995. Peranan tumbuhan dan ternak dalam upacara adat "Djoka Dju" pada suku Lio, Ende, Flores, Nusa Tenggara Timur. In: NASUTION, E. (Ed.). *Presiding Seminar dan Lokakarya Nasional Etnobotani II*. LIPI & Perpustakaan Nasional: 263-268. (In Indonesian).
SIMBOLON, H. & MIRMANTO, E. 2000. Checklist of plant species in the peat swamp forests of Central Kalimantan, Indonesia. In: IWAKUMA *et al.* (Eds.) *Proceedings of the International Symposium on: Tropical Peatlands*. Pp. 179-190.
- Book : RIDLEY, H. N. 1923. *Flora of the Malay Peninsula* 2. L. Reeve & Co. Ltd, London.
- Part of Book : BENTHAM, G. 1876. *Gesneriaceae*. In: BENTHAM, G. & HOOKER, J. D. *Genera plantarum* 2. Lovell Reeve & Co., London. Pp. 990-1025.
- Thesis : BAIRD, L. 2002. *A Grammar of Kéo: An Austronesian language of East Nusantara*. Australian National University, Canberra. [PhD. Thesis].
- Website : http://www.nationaalherbarium.nl/fmcollectors/k/Kostermans_AJGH.htm. Accessed 15 February 2012.



Reinwardtia

Published by Herbarium Bogoriense, Botany Division, Research Center for Biology,
Indonesian Institute of Sciences

Address: Jin. Raya Jakarta-Bogor Km. 46 Cibinong 16911, P.O. Box 25 Cibinong

Telp. (+ 62) 21 8765066; Fax (+62) 21 8765062

E-mail: reinwardtia@mail.lipi.go.id

REINWARDTIA Author Agreement Form

Title of article :

Name of Author(s) :

I/We hereby declare that:

- My/Our manuscript was based on my/our original work.
- It was not published or submitted to other journal for publication.
- I/we agree to publish my/our manuscript and the copyright of this article is owned by Reinwardtia.
- We have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in our article.

Author signature (s)

Date

Name

MUHAMMAD EFFENDI, TATIK CHIKMAWATI & DEDY DARNAEDI. New cytotypes of <i>Pteris ensiformis</i> var. <i>victoria</i> from Indonesia.....	133
SUZANA SABRAN, REUBEN NILUS, JOAN T. PEREIRA & JOHN BAPTIST SUGAU. Contribution of the heart of Borneo (HoB) initiative towards botanical exploration in Sabah, Malaysia.....	137
WENNI SETYO LESTARI, BAYU ADJIE, TASSANAI JARUWATANAPHAN, YASUYUKI WATANO & MADE PHARMAWATI. Molecular phylogeny of maidenhair fern genus <i>Adiantum</i> (Pteridaceae) from Lesser Sunda Islands, Indonesia based on <i>Rbcl</i> and <i>Trnl-f</i>	143
ELIZABETH A. WIDJAJA & DANIEL POTTER. Floristic study of Mekongga Protected Forest: towards establishment of the Mekongga National Park.....	157
YESSI SANTIKA, EKA FATMAWATI TIHURUA & TEGUH TRIONO. Comparative leaves anatomy of <i>Pandanus</i> , <i>Freycinetia</i> and <i>Sararanga</i> (Pandanaceae) and their diagnostic value.....	163
SUHARDJONO PRAWIROATMODJO & KUSWATA KARTAWINATA. Floristic diversity and structural characteristics of mangrove forest of Raj a Ampat, West Papua, Indonesia.....	171
IAN M. TURNER. A new combination in <i>Orophea</i> (Annonaceae) for <i>Uvaria nitida</i> Roxb. ex G. Don.....	181
IVAN S AVINOV. Taxonomic revision of Asian genus <i>Glyptopetalum</i> Thwaites (Celastraceae R. Br.).....	183
YUSI ROSALINA, NISYAWATL ERWIN NURDIN, JATNA SUPRIATNA & KUSWATA KARTAWINATA. Floristic composition and structure of a peat swamp forest in the conservation area of the PT National Sago Prima, Selat Panjang, Riau, Indonesia.....	193
IMAN HID AY AT & JAMJAN MEEBOON. <i>Cercospora brunfelsiicola</i> (Fungi, Mycosphaerellaceae), a new tropical Cercosporoid fungus on <i>Brunfelsia uniflora</i>	211
MAX VAN BALGOOY & ELIZABETH A. WIDJAJA. Flora of Bali: a provisional checklist.....	219
EKA FATMAWATI TIHURUA & INA ERLINAWATI. Leaf anatomy of <i>Pandanus</i> spp. (Pandanaceae) from Sebangau and Bukit Baka-Bukit Raya National Park, Kalimantan, Indonesia.....	223
JULIA SANG & RUTH KIEW. Diversity of <i>Begonia</i> (Begoniaceae) in Borneo - How many species are there?.....	23 3
DIAN LATIFAH, ROBERT A. CONGDON & JOSEPH A. HOLTUM. A Physiological approach to conservation of four palm species: <i>Arenga australasica</i> , <i>Calamus australis</i> , <i>Hydriastele wendlandiana</i> sa <i>Alicuala ramsayi</i>	237

REINWARDTIA
Vol. 14. No. 1.2014
CONTENTS
Page

ABDULROKHMAN KARTONEGORO & DANIEL POTTER. The Gesneriaceae of Sulawesi VI: the species from Mekongga Mts. with a new species of <i>Cyrtandra</i> described.....	1
LIM CHUNG LU & RUTH KIEW. <i>Codonoboea</i> (Gesneriaceae) sections in Peninsular Malaysia.....	13
WISNU H. ARDI, YAYAN W. C. KUSUMA, CARL E. LEWIS, ROSNIATI A. RISNA, HARRY WIRIADINATA, MELISSA E. ABDO & DANIEL C. THOMAS. Studies on <i>Begonia</i> (Begoniaceae) of the Molucca Islands I: Two new species from Halmahera, Indonesia, and an updated description of <i>Begonia holosericea</i>	19
YUZAMMI, JOKO R. WITONO & WILBERT L. A. HETTERSCHEID. Conservation status of <i>Amorphophallus discophorus</i> Backer & Alderw. (Araceae) in Java, Indonesia.....	27
MOHAMMAD F. ROYYANI & JOENI S. RAHAJOE. Behind the sacred tree: local people and their natural resources sustainability.....	35
FIFI GUS DWIYANTI, KOICHI KAMIYA & KO HARADA. Phylogeographic structure of the commercially important tropical tree species, <i>Dryobalanops aromatica</i> Gaertn. F. (Dipterocarpaceae) revealed by microsatellite markers.....	43
SACHIKO NISHIDA & HENK VAN DER WERFF. Do cuticle characters support the recognition of <i>Alseodaphne</i> , <i>Nothaphoebe</i> and <i>Dehaasia</i> as distinct genera?.....	53
NURUL AMAL LATIFF, RAHAYU SUKMARIA SUKRI & FAIZAH METALI. <i>Nepenthes</i> diversity and abundance in five habitats in Brunei Damssalam.....	67
NURUL HAZLINA ZATNI & RAHAYU SUKMARIA SUKRI. The diversity and abundance of ground herbs in lowland mixed Dipterocarp forest and heath forest in Brunei Darussalam.....	73
MUHAMMAD AMIRUL AIMAN AHMAD JUHARI, NORATNI TALIP, CHE NURUL ATNI CHE AMRI & MOHAMAD RUZI ABDUL RAHMAN. Trichomes morphology of petals in some species of Acanthaceae.....	79
DIAN ROSLEINE, EIZI SUZUKI, ATIH SUNDAWIATI, WARDI SEPTIANA & DESY EKAWATI. The effect of land use history on natural forest rehabilitation at corridor area of Gunung Halimun Salak National Park, West Java, Indonesia.....	85
JULIUS KULIP. The Ethnobotany of the Dusun people in Tikolod village, Tambunan district, Sabah, Malaysia.....	101
PETER O'BYRNE. On the evolution of <i>Dipodium R. Br.</i>	123

Reinwardtia is a LIPI accredited Journal (517/AU2/P2MI-LIPI/04/2013)

Herbarium Bogoriense
Botany Division
Research Center for Biology - Indonesian Institute of Sciences
Cibinong Science Center
Jln. Raya Jakarta - Bogor, Km 46
Cibinong 16911, P.O. Box 25 Cibinong
Indonesia