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ORIGINAL ARTICLE

Two fungi associated with necrotic leaflets of areca palms (*Areca catechu*)

**Chaiwat To-anun · Jeerapa Nguenhom ·
Jamjan Meeboon · Iman Hidayat**

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Abstract Information on fungi associated with necrotic leaflets of exotic palms in Thailand is scanty, as previous studies were mainly focused on decaying fronds. This study provides some information regarding fungi associated with necrotic leaflets of exotic palms in Thailand. Specimens in this work were collected from several locations in Chiang Mai province, including natural and planted areas. One new species, *Cercospora arecacearum* on areca palm (*Areca catechu*), is described and illustrated. An ITS sequence data analysis confirms the separation of the new *Cercospora* species from related taxa. Another species, *Neorehmia arecae*, hitherto only known from areca palm, is newly recorded for Thailand. Furthermore, *Wallichia siamensis* is a new host for this species.

Keywords Arecaceae · Biodiversity · Chiang Mai · Taxonomy · Tropical palmicolous fungi

Introduction

Fungi generally cause local and general necrosis of plant tissues, and they often cause reduced growth (stunting) of plant organs or entire plants (Agrios 2004). Leaf spot, blight, blotch, and anthracnose are common necrosis symptoms caused by fungi on plants. Necrosis on palm leaflets, such as leaf spot symptoms, can be caused by species of *Oxydothis* Penz. & Sacc., *Astrosphaeriella* Syd. & P. Syd., *Guignardia* Viala & Ravaz, *Maculatipalma* J. Fröhl. & K.D. Hyde, and *Mycosphaerella* Johanson; however, most of the reported taxa were examined from specimens of palms in Australia and Papua New Guinea (Fröhlich 1992; Fröhlich and Hyde 1994, 1995a, b, c, 1998, 2000; Hyde and Fröhlich 1995; Hyde et al. 1997).

Many new species of several palmicolous genera have been reported in Thailand during the last approximately 10 years, but specific reports on fungi associated with necrotic leaflet of palms are few, as the previous studies mainly focused on decaying fronds (Aramsiriujiwet 1996; Hidayat et al. 2006; Hyde et al. 2002; McKenzie et al. 2002; Pinnoi et al. 2004, 2006; Pinruan et al. 2002, 2004; Sarapat 2003). Furthermore, fungi potentially causing necrotic spots are particularly important on economically used palms, like the areca palm (*Areca catechu*). Therefore, we began to survey the diversity of this group of fungi, particularly in a highland forest area of the northern part of Thailand, wherein several species of tropical palms are distributed. In this paper, we report two interesting fungi associated with necrotic leaflet of palms from this area, including one new species.

Materials and methods

Collection sites and morphological examination

Specimens collected in this study came from several natural and planted sites in Chiang Mai province. One or two 29× 42 cm resealable plastic bags were used for each palm species. On returning to the laboratory, the material was

C. To-anun · J. Nguenhom · J. Meeboon · I. Hidayat (✉)
Department of Plant Pathology, Chiang Mai University,
Chiang Mai, Thailand
e-mail: imanhidayat@yahoo.com

I. Hidayat
Microbiology Division, Research Center for Biology,
Indonesian Institutes of Sciences,
Bogor, Indonesia

examined immediately and the remains were incubated in a moist chamber for studying at a later date.

A sharp one-sided razor blade was used to carefully remove the tops of the fruiting bodies of the fungal specimens belonging to Ascomycetes. If the contents were wet, they were extracted using a pair of Inox 5 fine forceps. If the contents were dry or crystalline, they were rehydrated using distilled water or 5% KOH before extraction. Ascomata were crushed in a drop of water on a glass slide and then covered with a coverslip to observe asci, ascospores, and paraphyses/pseudoparaphyses. Hyphomycetes were treated by hand sectioning or using a pair of Inox 5 fine forceps to carefully remove conidiophores and conidia from the host.

The specimens were observed using an Olympus BX50 photomicroscope system with differential interference contrast microscopy. Water was the medium used for all examinations, spore measurements, and most of the illustrations. Measurements are given as (minimum) mean ± standard deviation (maximum) (n=sample size). Specific reagents were used when necessary as follows: Melzer's reagent was used to investigate any reactions in the ascus; Ascomata sections of rehydrated fruiting structures were made with a Micron HM505E cryostat microtome or by hand; and Lactophenol was added to the slides for permanent fixation. Dried herbarium specimens were deposited at Mushroom Research Center Herbarium, Chiang Mai, Thailand, and CMU Herbarium (CMU), Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. Cultures were obtained in this study according to the method of Choi et al. (1999). An ex-type culture is maintained in the Molecular of Plant Pathology Culture Collection, Department of Plant Pathology, Chiang Mai University, Chiang Mai, Thailand and BIOTEC culture collection (BCC), Pathumthani, Thailand.

Molecular characterization

In this study, molecular characterization of the isolate of the new species was carried out in order to analyze its phylogenetic relationship with other similar taxa. Total genomic DNA was extracted from mycelial cultures grown on malt extract agar (Difco) following a 2× cetyltrimethylammoniumbromide (CTAB) protocol (Cai et al. 2005). DNA amplification of internal transcribed spacer (ITS) nrDNA region was performed by polymerase chain reaction (PCR) using ITS4 and ITS5 primers (White et al. 1990) to generate about 587 nucleotides from the complete ITS region. The amplification conditions were performed in a 50-µl reaction volume as follows: 1×PCR buffer, 0.2 mM each dNTP, 0.3 mM of each primer, 1.5 mM $MgCl_2$, 0.8 units Taq polymerase, and 10 ng DNA. PCR parameters for all the regions were performed as follows: initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 1 min, 52°C for 50 s, and 72°C for 1 min, and final extension of 72°C for 10 min.

The characterization of PCR products was performed via agarose gel electrophoresis on a TAE 1% agarose gel containing ethidium bromide (EtBr) as the staining agent. The PCR product was purified using Qiaquick purification kit (Qiagen) and DNA concentration of the PCR products was subjected to automatic sequencing (ABI PRISM Dye Terminator Cycle Sequencing and ABI PRISM Sequencer model 377; Perkin Elmer). The new ribosomal DNA sequence has been deposited in GenBank under accession number EU581822. (The GenBank accession numbers of the other sequences and taxa used to construct the phylogenetic trees are shown in Fig. 2 below.)

Sequences were aligned in ClustalX version 2.0.3 (Larkin et al. 2007) and BioEdit (Hall 1999) using default parameters. The sequences alignments were checked and manual adjustments were made where necessary. Regions designated as ambiguously aligned were excluded from the analyses. Gaps were treated as missing data. The alignments were deposited in TreeBASE (http://treebase.org/) under the accession number of SN3932. Phylogenetic analyses were performed in PAUP version 4.0b10 (Swofford 2002).

Unweighted Maximum Parsimony (UMP) analysis was performed in this study. Trees were inferred using the heuristic search option with 1,000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics [tree length (TL), consistency index (CI), retention index (RI), related consistency index (RC), homoplasy index (HI), and log likelihood (-ln L)] were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa (KH) likelihood test (Kishino and Hasegawa 1989) was carried out using PAUP to compare the best tree topology obtained by the nucleotide sequence data with a constrained tree. Clade stability was assessed in bootstrap analyses with 1,000 replicates, each with 1,000 replicates of random stepwise addition of taxa. Random sequence addition was used in the bootstrap analyses. Trees were figured in TreeView (Page 1996). Other details are outlined in Cai et al. (2005).

Results and discussion

Taxonomy

Cercospora arecacearum Hidayat & Meeboon, sp. nov. fig. 1(a–e)

MycoBank No. MB 510616

Differt a *C. apii* s. lat. (*C. nucifera*), *C. palmae-amazonensis* et *C. raphiae* stromatibus bene evolutis, (30) 64±26 (100) µm diam., conidiophoris raro ramosis, multi-

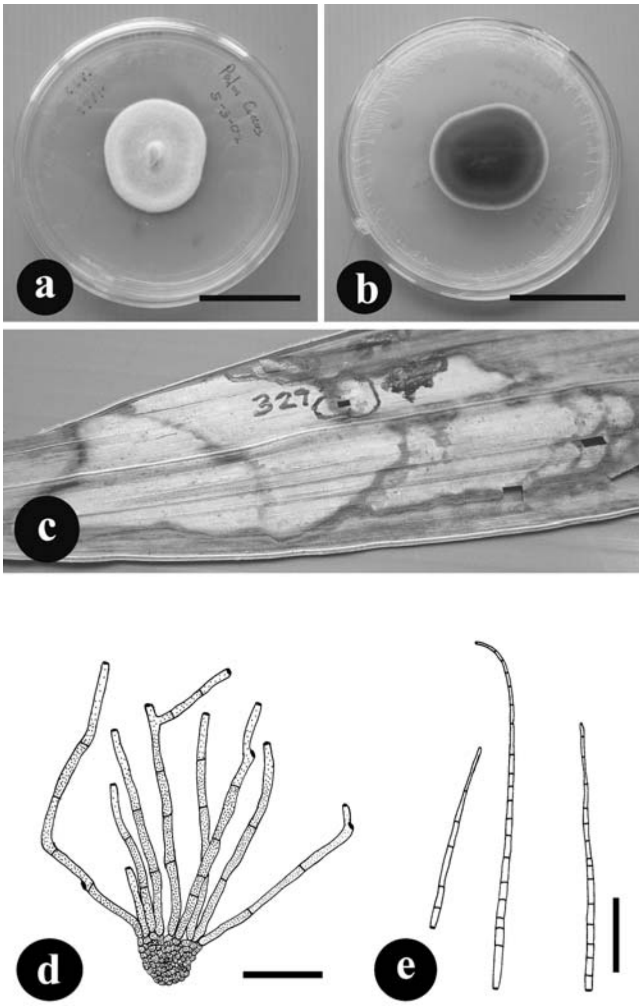

Fig. 1 *Cercospora arecacearum* (from holotype). **a** Culture on the upper surface on PDA, **b** culture on the lower surface on PDA, **c** symptoms on the upper surface of the host, **d** conidiophores and stroma, and **e** conidia. *Scale bars* **a** 3.5 cm, **b**4 cm, **d**75 µm, **e** 60 µm

geniculatis et a *C. palmae-amazonensis* et *C. raphiae* conidiis acicularibus.

Etymology: Derived from Arecaceae, the host family of this species.

Leaf spots amphigenous, irregular, 1–10 cm diam., brownish, dull grayish brown, finally pale grayish with a white center and dark margins, spots usually overlapping. Caespituli amphigenous, scattered, and dark yellowish. Stromata substomatal to intraepidermal, well-developed, subglobular, brown to blackish brown, (30) 64±26 (100) µm diam. (*n*=10). Conidiophores in rich fascicles, dense, arising from stromata, cylindrical, but narrowed towards the apex, straight, rarely branched, strongly geniculate, variable in length, (68.5) 165±91 (310) × (4) 4.5±0.5 (5) µm (*n*=30), smooth, pale yellowish to brownish throughout, sometimes paler at the apex, 2–8-septate. Conidiogenous cell integrated, terminal, sympodially proliferating, (24.5) 37±13 (67) × (4) 4±0.4 (5) µm (*n*=30). Conidiogenous loci conspicuous, thickened and darkened, (2.5) 3±0.3 (3) µm diam. (*n*=30). Conidia formed singly, acicular, straight, often curved at the apex, (140) 229±56 (320) × (4) 5±0.5 (5) µm (*n*=30), hyaline, 9–25-septate, thin-walled, smooth, tapered towards a subacute apex, base truncate, hilum thickened and darkened, (2.5) 3±0.3 (3) µm diam (*n*=30).

On potato dextrose agar medium: colony slowly growing, velvety, 3–4 cm after 30 days, tight to the agar, dark, covered by a grayish white aerial mycelium, reddish near the margin, with white margin, producing red pigmentation in the agar, no sporulation.

Holotype: THAILAND, Chiang Mai province, Mae Taeng district, Pa Pae village, Mushroom Research Centre, on leaf spots of *Areca catechu* L. (Arecaceae), 17 November 2006, Iman Hidayat (CMU 27946). Ex-type culture (BCC 32698).

Habitat: Leaf spots of *Areca catechu* (Arecaceae).

Distribution: Chiang Mai, Thailand.

Remarks: According to Crous and Braun (2003), this species belongs to *Cercospora* Fresen. s. str., which is characterized by having pigmented conidiophores, thickened and darkened conidiogenous loci, and hyaline scolecoid conidia. Furthermore, this fungus is distinct from the plurivorous *C. apii* s. lat. by having well-developed, large stromata, and strongly geniculate, rarely branched conidiophores in rich fascicles (Crous and Braun 2003).

About 12 species of *Cercospora* s. lat. are hitherto known on Arecaceae. Most of them have been excluded from *Cercospora* s. str. based on comprehensive re-examinations by several researchers (Anderson et al. 2000; Chupp 1954; Crous and Braun 2003; Deighton 1985; Goh and Hsieh 1989; Hughes 1952). Hughes (1952) excluded *C. palmivora* Sacc. (= *C. preisii* Bubák) from *Cercospora* and put it in *Stigmina* as *S. palmivora* (Sacc.) S. Hughes. Chupp (1954) excluded three additional species of *Cercospora* on Arecaceae by transferring *C. acrocomiae* J.A. Stev. to *Exosporium* Link, *C. calamicola* Henn. to *Helminthosporium* Link, and by classifying *C. licualae* Syd. & P. Syd. (= *Cercospora virens* Sacc.) as helminthosporoid fungus. Deighton (1985) assigned *C. elaeidis* Steyaert to *Pseudospiropes* due to the type of lesions and pigmented conidia. Anderson et al. (2000) re-examined the type material of *C. palmicola* Speg. and renamed this fungus as *Drechslera palmicola* (Speg.) Anderson, Bianchinotti & U. Braun due to its tretic conidiogenesis, with wide and plainly thick-walled conidia. Goh and Hsieh (1989) introduced the new combination, *Pseudocercospora rhapisicola* (Tominaga) Goh & W.H. Hsieh, for *C. rhapisicola* Tominaga because of inconspicuous, unthickened conidial scars and hila.

Currently, only two species on Arecaceae, viz, *Cercospora palmae-amazonensis* Bat. & Cavalc. (Batista and Cavalcanti 1964) and *C. raphiae* Deighton (1985), have been maintained in *Cercospora* s. str. (Crous and Braun 2003). Another species of *Cercospora* s. str., *C. nucifera* R.K. Srivast., S. Narayan and A.K. Srivast. (Srivastava et al. 1995), is now classified as *C. apii* s. lat. (Crous and Braun 2003).

Cercospora arecacearum is distinct from *C. raphiae* by having amphigenous caespituli, rarely branched and strongly geniculate conidiophores as well as much narrower acicular conidia. Deighton (1985) characterized *C. raphiae* by having hypophyllous caespituli, unbranched, non-geniculate conidiophores and obclavate-cylindrical conidia with slightly thickened hila.

Batista and Cavalcanti (1964) described *C. palmae-amazonensis* as having amphigenous caespituli, conidiophores in fascicles, oblong stromata (22–25 µm diam.), unbranched, simple, to slightly geniculate conidiophores (6–10 septate, 112–250×4–5 µm), and lightly brown obclavate conidia with truncate base (6–9 septate, 40–90×2.5–7.5 µm). The taxonomic affinity of this species remains uncertain due to the described pigmentation of the conidia. Crous and Braun (2003) examined authentic material of this species, but failed to find any fructification. *Cercospora arecacearum* is easily distinguishable from *C. palmae-amazonensis* by its large stromata, branched, and strongly geniculate conidiophores with hyaline acicular conidia (Table 1).

Molecular phylogenetic analysis of ITS DNA region of *C. arecacearum* was carried out in this study in order to confirm the morphological elucidation of the fungus with related taxa, particularly members of true cercosporoid fungi sensu Crous and Braun (2003). The ITS sequence of *C. arecacearum* was aligned with 34 sequences of species of *Cercospora*, *Pseudocercospora* Speg., and *Passalora* Fr. Sequences of *Cladosporium cladosporioides* (Fresen.) G.A. de Vries and *Davidiella tassiana* (De Not.) Crous & U. Braun were assigned as outgroup. The alignment data matrix consists of 35 taxa and 527 characters, of which 38 characters were excluded from the analysis due to ambiguity of the alignment. Of the remaining 489 included characters, 329 characters were constant, 45 characters were variable and parsimony-uninformative, and 115 characters were parsimony-informative. Sum of minimum possible length was 207 and sum of maximum possible length was 729. The best parsimonious tree selected by KH test ($P<0.05$) was generated in 264 steps (CI=0.750, RI=0.868, RC=0.651, HI=0.250).

Based on this analysis, three well-supported clades (*Cercospora*, *Pseudocercospora*, and *Passalora*) were generated with bootstrap values greater than 75% for each clade. These three clades, which are defined as true cercosporoid fungi by Crous and Braun (2003), formed a monophyletic clade with 100% bootstrap support. The characteristics among these three clades were elucidated morphologically and phylogenetically by Stewart et al. (1999). The *Pseudocercospora* clade appeared as a sister group to the *Cercospora* clade with 80% bootstrap support, and *Passalora* clade appeared as a sister group to the *Cercospora* and *Pseudocercospora* clades with 100% bootstrap support. All the *Cercospora* sequences showed a distinct and monophyletic clade with a well-supported bootstrap support value (95%). In this clade, *C. arecacearum* together with non-*Cercospora apii* s. lat. taxa such as *C. acaciae-mangii* Crous, Pongpan. & M.J. Wingf., *C. agavicola* Ayala-Escobar, *C. asparagi* Sacc., *C. caricis* Oudem., *C. piaropi* Tharp, *C. polygonaceae* Ellis & Everh., *C. rodmanii* Conway, and *C. sorghi* Ellis & Everh. f. *maydis* Ellis & Everh., clustered alongside the *C. apii* s. lat. taxa with a bootstrap support value 62%. On the other hand, only *C. zeae-maydis* Tehon & E.Y. Daniels was separated from this clade. Therefore, these data suggested that *Cercospora* species belonging to *C. apii* s. lat. do not generate a monophyletic clade based on the phylogenetic analysis of ITS sequence data. In conclusion, *C. arecacearum* is considered a new cercosporoid species, which is morphologically distinct from other similar taxa, and phylogenetically separated from related taxa (Fig. 2).

Neorehmia arecae (Syd.) Samuels & M.E. Barr

Material exmained: THAILAND, Chiang Mai Province, Doi Suthep-Pui National Park, Huay Kog Ma, on necrotic

Table 1 Morphological comparison of *Cercospora arecacearum* with other *Cercospora* species from Arecaceae. (Data from Batista and Cavalcanti 1964, Deighton 1985)

Morphological characteristics	*C. arecacearum*	*C. palmae-amazonensis*	*C. raphiae*
Leaf spots	Amphigenous	Amphigenous	Amphigenous
Colonies	Amphigenous	Amphigenous	Hypophyllous
Stromata	Well-developed, (30) 64±26 (100) µm diam.	Small, 22–25 µm diam.	Well-developed, 25–40 µm diam.
Conidiophores	Densely fasciculate, strong geniculate, rarely branched, (68.5) 165±91 (310) × (4) 4.5± 0.5 (5) µm	Densely fasciculate, simple to slightly geniculate, unbranched, 112–250×4–5 µm	Divergent fasciculate, simple to slightly geniculate, unbranched, up to 210×6.5 µm
Conidia	Solitary, acicular, hyaline, (140) 229±56 (320) × (4) 5±0.5 (5) µm	Solitary, obclavate, pigmented, 40–90×2.5–7.5 µm	Solitary, obclavate-cylindric, hyaline, 48–117×5.5–8 µm

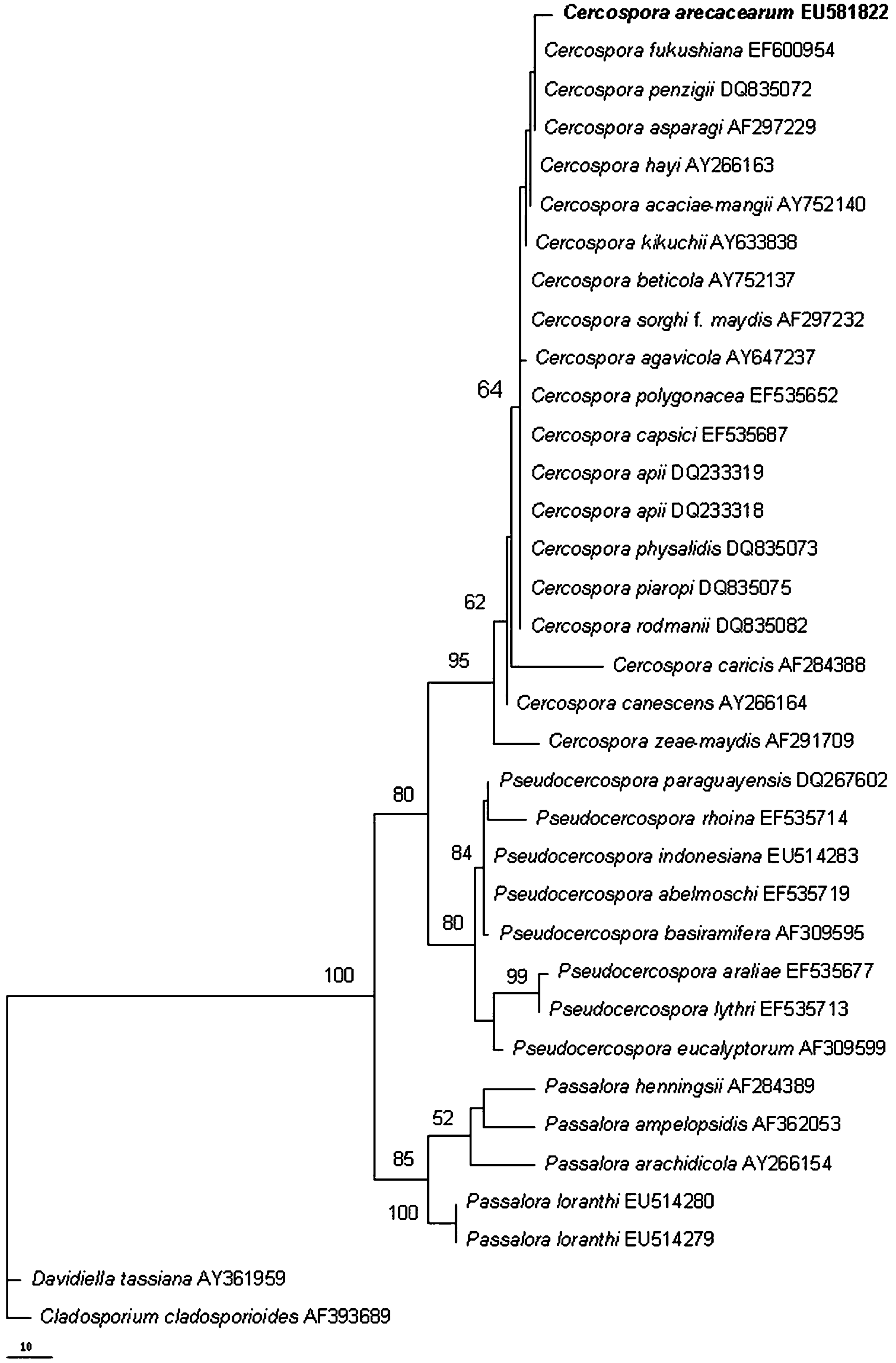

Fig. 2 Single parsimonious tree based on ITS nrDNA sequence data representing placement of *Cercospora arecacearum* within representatives of the family Mycosphaerellaceae. The tree is obtained from heuristic search with 1,000 random taxon addition of the sequences alignment. Bootstrap values (>50%) from 1,000 replicates of Unweighted Maximum Parsimony (UMP) analyses are shown above internodes

leaflet of *Wallichia siamensis* Becc. (Arecaceae), 16 October 2005, Iman Hidayat (Mushroom Research Center Herbarium 00202); 25 October 2006, Iman Hidayat (CMU 27950).

Remarks: *Neorehmia arecae* (Syd.) Samuels & M.E. Barr (Trichosphaeriaceae, Trichosphaeriales) [≡ *Trichosphaerella arecae* Syd.], an ascomycetous fungus on leaf spot of areca palms, was proposed by Samuels and Barr (1997) based on the similarity of morphological characteristics to those of *N. ceratophora* Höhn. (type species) which is characterized by setae which are branched at the tips. The part spores of *N. ceratophora* are subglobose, smooth-walled, and numerous; narrow and delicate paraphyses are usually seen interspersed among the asci. *Neorehmia* is also connected to a *Tritirachium*-like anamorph by Müller and Samuels (1982) whereas *Trichosphaerella* was linked to an *Acremonium*-like anamorph.

The specimen examined here is typical of *N. arecae* due to flattened and sphaeroid fruit-bodies with a few short setae branching at the tip, the part spores which are subglobose, and also by being recorded from a palm (Müller and Dennis 1965; Samuels and Barr 1997). This specimen is the second report of *N. arecae* worldwide and the first record in Thailand. Furthermore, *Wallichia siamensis* is a new host of this species.

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References

Agrios GN (2004) Plant pathology, 5th edn. Elsevier, Oxford

Anderson F, Bianchinotti MV, Braun U (2000) *Drechslera palmicola* comb. nov. and *Exosporium puccinioides* comb. nov. Schlechtendalia 5:67–69

Aramsiriujiwet Y (1996) Hyphomycetes on bamboo and Palmae in Thailand. MSc thesis, Kasetsart University, Thailand

Batista AC, Cavalcanti WA (1964) *Cercospora*: duas novas especies do Amazonas e da bahia. Anais do XII Congresso da Sociedade Botânica do Brasil (Janeiro-1962), pp 385–388

Cai L, Jeewon R, Hyde KD (2005) Phylogenetic evaluation and taxonomic revision of *Schizothecium* based on ribosomal DNA and protein coding genes. Fungal Divers 19:1–17

Choi YW, Hyde KD, Ho WH (1999) Single spore isolation of fungi. Fungal Divers 3:29–38

Chupp C (1954) A monograph of the fungus genus *Cercospora*. Published by the author, Ithaca, New York, USA

Crous PW, Braun U (2003) *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Series 1. CBS, Utrecht, The Netherlands, 571 pp

Deighton FC (1985) Three leaf-spotting hyphomycetes on palms. Trans Br Mycol Soc 85:739–742

Fröhlich J (1992) Fungi associated with foliar diseases of palms in north Queensland. BSc thesis, School of Botany, Melbourne University, Melbourne, Australia

Fröhlich J, Hyde KD (1994) New *Oxydothis* species associated with palm leaf spots in north Queensland, Australia. Mycol Res 98:213–218

Fröhlich J, Hyde KD (1995a) *Astrosphaeriella frondicola* sp. nov. associated with leaf spots on *Oraniopsis* and other palms. Mycol Res 99:453–459

Fröhlich J, Hyde KD (1995b) *Guignardia candeloflamma* sp. nov. causing leaf spot of *Pinanga* sp. Mycol Res 99:110–112

Fröhlich J, Hyde KD (1995c) *Maculatipalma frondicola* gen. et sp. nov. causing leaf spots on palm species in north Queensland with descriptions of related genera: *Apioplagiostoma* and *Plagiostoma*. Mycol Res 99:727–734

Fröhlich J, Hyde KD (1998) Fungi from palms. XXXVIII. The genera *Mycosphaerella* and *Sphaerella*. Sydowia 50:171–181

Fröhlich J, Hyde KD (2000) Palm microfungi. Fungal diversity research series 3. Fungal Diversity Press, Hong Kong SAR, PR China 375 pp

Goh TK, Hsieh WH (1989) Studies on *Cercospora* and allied genera of Taiwan (VIII). New species and new combinations. Trans Mycol Soc Repub China 4:39–56

Hall TA (1999) BioEdit: a user—friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. Nucleic Acids Sympo Ser 41:95–98

Hidayat I, Jeewon R, To-anun C, Hyde KD (2006) The genus *Oxydothis*: new palmicolous taxa and phylogenetic relationship within Xylariales. Fungal Divers 23:159–179

Hughes SJ (1952) Studies on micro-fungi. XIV. *Stigmella*, *Stigmina*, *Camptomeris*, *Polythrincium*, and *Fusicladiella*. Mycol Pap 49:1–25

Hyde KD, Fröhlich J (1995) *Mycosphaerella palmicola* associated with leaf spots of *Cocos nucifera* in Australia, Irian Jaya and Papua New Guinea. Mycol Res 99:704–706

Hyde KD, Fröhlich J, Taylor JE (1997) Diversity of ascomycetes on palms in the tropics. In: Hyde KD (ed) Biodiversity of tropical microfungi. Hong Kong University Press, Hong Kong, SAR, PR China, pp 141–156

Hyde KD, Yanna, Pinnoi A, Jones EBG (2002) *Goidanichiella fusiforma* sp. nov. from palm fronds in Brunei and Thailand. Fungal Divers 11:119–122

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. J Mol Evol 29:170–179. doi:10.1007/BF02100115

Larkin MA, Blackshields G, Brown NP, Chenna R, McTgettigan H, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. doi:10.1093/bioinformatics/btm404

McKenzie EHC, Pinnoi A, Wong MKM, Hyde KD, Jones EBG (2002) Two new hyaline *Chalara* and key to species described since 1975. Fungal Divers 11:129–139

Müller E, Dennis RWG (1965) Fungi venezuelani: VIII. Plectascales, sphaeriales, loculoascomycetes. Kew Bull 19:357–386. doi:10.2307/4108161

Müller E, Samuels GJ (1982) Anamorphs of pyrenomycetous ascomycetes I. *Rhamphoria* Niessl and *Trichosphaerella* Bommer, Rousseau & Saccardo. Sydowia 35:143–149

Page RDM (1996) TreeView, an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Pinnoi A, Pinruan U, Hyde KD, Lumyong S, Jones EBG (2004) Palm fungi. In: Jones EBG, Tanticharoen M, Hyde KD (eds) Thai fungal diversity. BIOTEC, Bangkok, Thailand, pp 181–187

Pinnoi A, Lumyong S, Hyde KD, Jones EBG (2006) Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiwat, Thailand. Fungal Divers 22:205–218

Pinruan U, Jones EBG, Hyde KD (2002) Aquatic fungi from peat swamp palms: *Jahnula appendiculata* sp. nov. Sydowia 54:242–247

Pinruan U, Lumyong S, McKenzie EHC, Jones EBG, Hyde KD (2004) Three new species of *Craspedodidymum* from palm in

Thailand. Mycoscience 45:177–180. doi:10.1007/s10267-003-0173-5

Samuels GJ, Barr ME (1997) Notes on and additions to the Niessliaceae (Hypocreales). Can J Bot 75:2165–2176

Sarapat T (2003) Diversity of Ascomycetes and mitosporic fungi on Palmae in Siridhorn swamp forest ecosystem, Narathiwat Province, Thailand. MSc thesis, Prince of Songkla University, Songkhla, Thailand

Srivastava RK, Narayan S, Srivastava AK (1995) *Cercospora* species causing leaf spots in crops and horticultural plants of north-eastern Uttar Pradesh. Indian Phytopathol 48:106–107

Stewart EL, Liu ZW, Crous PW, Szabo LJ (1999) Phylogenetic relationships among some cercosporoid anamorphs of *Mycosphaerella* based on rDNA sequence analysis. Mycol Res 103:1491–1499. doi:10.1017/S0953756299008680

Swofford DL (2002) PAUP*: phylogenetic analyses using parsimony (* and other methods), version 4.0b 10. Sinauer Associates, Sunderland, Massachusetts

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and application. Academic Press, San Diego, pp 315–322