Phytotaxa 88 (3): 49–54 (2013)
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.88.3.2

A new species of *Pestalotiopsis* from leaf spots of *Licuala grandis* from Hainan, China

KUN GENG[1,2,3], BIN ZHANG[3], YU SONG[1], KEVIN D. HYDE[4,5], JI-CHUAN KANG[2] & YONG WANG[1]

[1] *Department of Plant Pathology, Agriculture College, Guizhou University, 550025, China*
email: yongwangbis@yahoo.cn
[2] *Guizhou Biochem-Engineering Research Center, Guizhou University, 550025, China*
email: bcec.jckang@gzu.edu.cn
[3] *Plant Protection and Quarantine Station, Guiyang City, 550081, China*
[4] *Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[5] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*

Abstract

A new species, *Pestalotiopsis licualacola*, was isolated from grey leafspots of *Licuala grandis* (ruffled fan palm). It is morphologically distinct in having relatively small, greyish brown conidia (16–20 × 3–5 µm), and 1–3 short apical appendages without knobs. Phylogenetic analysis based on combination of ITS, β-tubulin and *tef1* gene sequence data clearly distinguishes *P. licualacola* from other species in this genus, with ex-type sequence data in GenBank. Based on morphology and molecular phylogeny we describe it as a new species.

Key words: coelomycetes, phylogeny, taxonomy

Introduction

The ruffled fan palm (*Licuala grandis*) is a dainty palm with a height of approximately 2 m that originates in South East Asia, and is a rainforest understory palm (www.nationaltropicals.com.au/). During investigations of *Pestalotiopsis* in southern China, we have obtained many isolates of *Pestalotiopsis* spp. from diseased leaves of diverse plants, which we are using for novel compound screening as the genus is biochemically highly creative (Xu *et al.* 2010, Aly *et al.* 2011, Debbab *et al.* 2011, 2012). Among them was an undescribed species isolated from necrotic leaf spots from *L. grandis*. Morphological details are described and a comparison made with related species. Molecular characteristics based on the DNA sequences of three gene loci (ITS, β-tubulin and *tef1*) were also determined.

Materials & Methods

Morphological and cultural studies

Diseased leaves of ruffled fan palm were collected from Xinglong County, Hainan Province. Leaf samples were placed in clean paper bags and symptoms were recorded. A single conidium culture technique was preformed to obtain pure colonies of the fungi following the method outlined in Chomnunti *et al.* (2011). The colonies were transferred to 2% potato-dextrose agar (PDA) medium and incubated at room temperature (25°C). Sporulation was induced using sterilized carnation leaves, which were aseptically placed on the surface of the medium with growing mycelium. The morphology of fungal colonies was recorded following the method of Hu *et al*. (2007). Fungal mycelium and spores were observed under the light microscope

Accepted by Eric Mckenzie: 5 Mar. 2013; published online in PDF: 22 Mar. 2013

(Nikon 80i) and photographed. Methods of examination, photography and isolation followed Boonmee *et al.* (2011). Dried and ex-type cultures of this species are deposited in the Plant Pathology Herbarium of Guizhou University (HGUP).

DNA sequencing and alignment

Total genomic DNA was extracted from fresh cultures using a modified protocol of Doyle & Doyle (1987) and Lee & Taylor (1990). The ITS and 5.8S region of rDNA molecule was amplified using primer pairs ITS4 and ITS5 (White *et al*. 1990); β-tubulin gene region was amplified with primer pairs BT2A and BT2B (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997); and *tef1* was amplified using the primer pairs EF1-526F and EF1-1567R (Rehner 2001). PCR was performed with the 25 μL reaction system consisting of 19.75 μL of double distilled water, 2.5 μL of 10× Taq buffer with $MgCl_2$, 0.5 μL of dNTP (10 mM each), 0.5 μL of each primer (10 μM), 0.25 μL Taq DNA polymerase (5 U/μl), 1.0 μL of DNA template. The thermal cycling program followed Maharachchikumbura *et al*. (2012). The DNA sequences of HGUP4057 in ITS, beta-tubulin and *tef1* regions generated in this study were submitted to GenBank (KC436006, KC481683 and KC481684).

Phylogenetic analyses

Combination sequence data obtained from three gene regions (ITS, β-tubulin and *tef1*) were aligned using CLUSTALX (v. 1.83) (Thompson *et al*. 1997). The sequences were manually adjusted using BioEdit (Hall 1999), to allow maximum alignment and maximum sequence similarity. Alignments file is available in TreeBASE (www.treebase.org/treebase–web/home.html) with study ID 13925. A maximum parsimony analysis (MP) was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees were set up to 5,000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], and homoplasy index [HI] were determined. The robustness of the most parsimonious trees was evaluated by 100 bootstrap replications resulting from maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether the trees inferred under different optimality criteria were significantly different.

Results

Phylogenetic analysis

DNA sequences from our *Pestalotiopsis* isolate were compared with those originated from Maharachchikumbura *et al*. (2012) with *Seiridium* sp. (SD096) as outgroup. The alignment file resulted in a data set comprising 2051 characters including gaps. Of these characters, 1434 were constant and parsimony-uninformative. The 617 parsimony-informative characters included in the parsimonious analyses yielded 18 parsimonious trees (TL = 1514, CI = 0.682, RI = 0.884, RC = 0.603), one of which was selected to represent the topology of the strict consensus tree (Fig. 1). Forty-five *Pestalotiopsis* isolates (27 taxa) formed a large clade with 100% bootstrap support, which was divided into subclades A and B with 65% and 97% bootstrap values, respectively. Our isolate (HGUP4057) clustered in subclade A with *P. trachicarpicola*, *P. rosea* and *P. adusta*, with a high bootstrap value of 95%. HGUP4057 showed a close relationship with two *P. adusta* isolates (ICMP6088 – epitype culture of *P. adusta* and MFLUCC10-146) supported by a strong bootstrap value (96%).

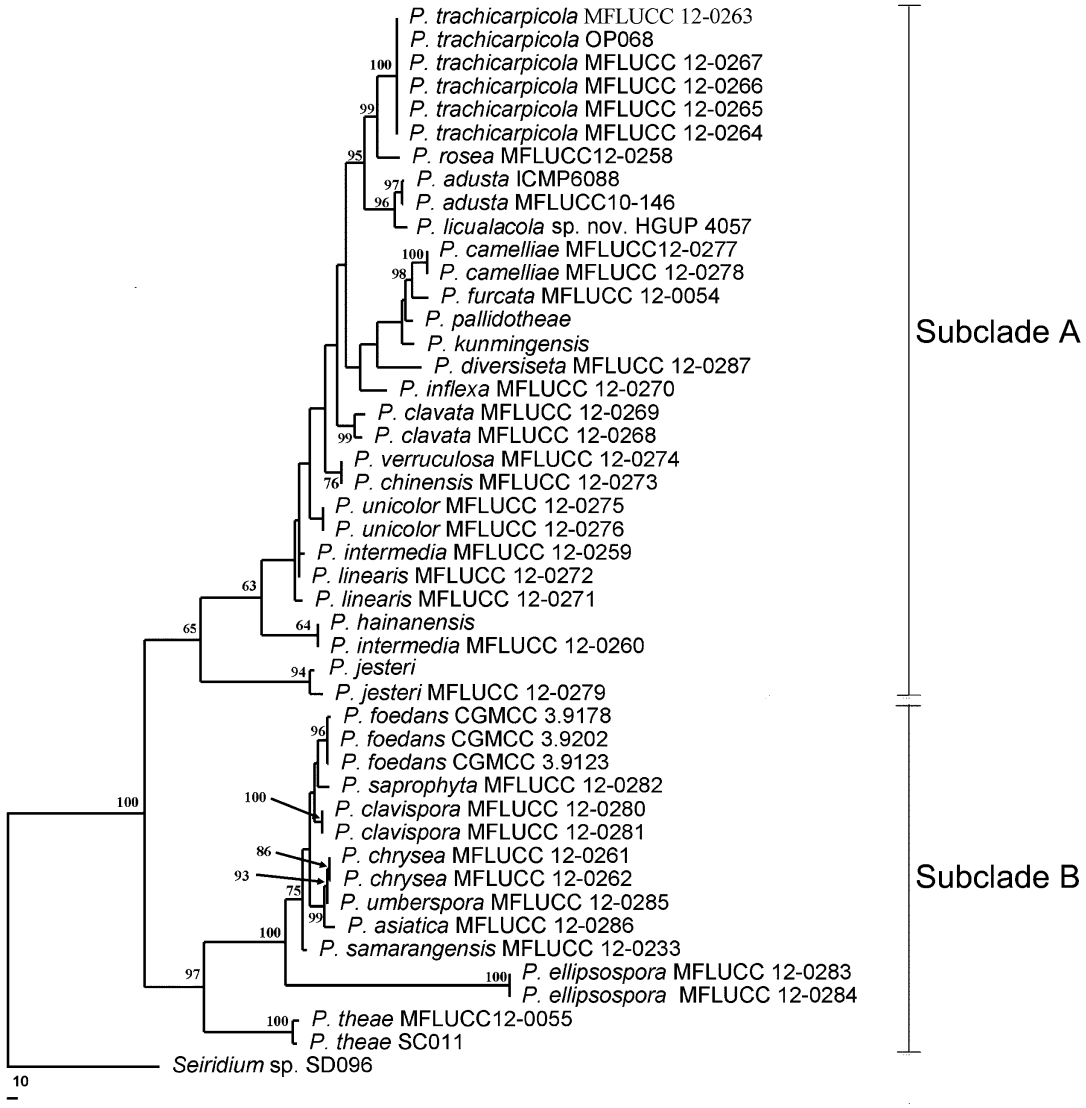

FIGURE 1. Topology showing the most parsimonious tree, inferred from combined ITS, β -tubulin and *tef1* gene regions. Bootstrap values smaller than 50% are not shown. The tree was rooted with *Seiridium* sp. (SD096).

Taxonomy

Pestalotiopsis licualacola K. Geng, Y. Song, K.D. Hyde & Yong Wang bis, *sp. nov.* (Fig. 2) MycoBank MB 803183

Type:—CHINA. Hainan Province: Xinglong County, Tropical Botanical Garden, living leaves of *Licuala grandis*, 8 March 2012, HGUP4057, *K. Geng*, HGUPd4057, holotype!

Differs from related *Pestalotiopsis* and *Pestalosphaeria* species mainly by its noticeably narrower, fusiform conidia with mostly a single apical appendage.

Colonies on PDA attaining 7 cm diam. after 7 days at 25°C, with edge undulate, whitish, aerial mycelium on surface, fruiting bodies black, concentric; reverse of culture yellow to pale brown. Conidiophores most often indistinct. Conidiogenous cells discrete, hyaline, simple, filiform, 4–10 μm long. Conidia 16–20 × 3–5 μm ($\overline{x}$ = 17.4 × 3.9 μm), fusiform, straight to slightly curved, 4-septate, smooth, greyish brown; basal cell conical, hyaline, thin-walled, 2–4 μm long ($\overline{x}$ = 2.4 μm); with three median cells,

dark brown, concolorous, septa and periclinal walls darker than the rest of the cell, together 9.5–12 μm long ($\overline{x}$ = 11 μm); second cell from base 2.7–4.2 μm ($\overline{x}$ = 3.6 μm); third cell 2.4–4 μm ($\overline{x}$ = 3.3 μm); fourth cell 2.5–3.8 μm ($\overline{x}$ = 3.2 μm); apical cell hyaline, conic to subcylindrical, 1.8–3.6 μm ($\overline{x}$ = 2.4 μm); with 1–3 tubular apical appendages (mostly 1) without knobs, arising from the apex of the apical cell, 4–9.5 μm long ($\overline{x}$ = 6.6 μm); basal appendage filiform, short.

Etymology: —In reference to the host, *Licuala grandis*, from which this fungus was first isolated.

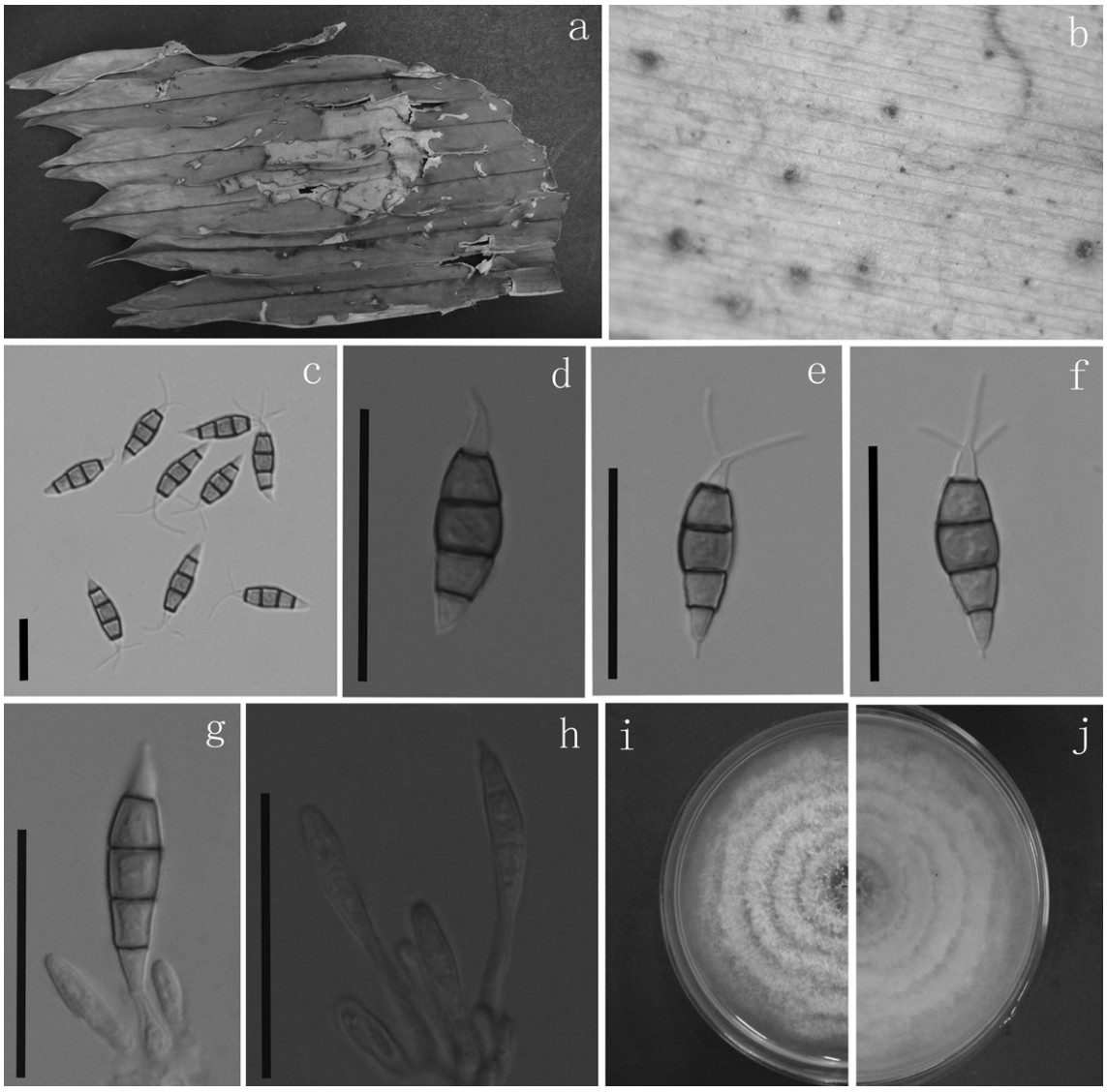

FIGURE 2. *Pestalotiopsis licualacola* (holotype). a. Herbarium material, leaves of *Licuala grandis*. b. Conidiomata. c–f. Conidia with concolorous median cells. g–h. Conidiophores/conidiogenous cells. i–j. Colony on PDA, i. from above, j. from below. Scale bars: c–h = 20 μm.

Discussion

It is important to clarify relationships amongst *Pestalotiopsis* species as they are weak pathogens, but also prolific producers of novel medicinal compounds (Xu *et al*. 2010, Aly *et al*. 2011, Ko Ko *et al*. 2011a, b, Debbab *et al*. 2011, 2012). Several *Pestalotiopsis* species are known from palms and perhaps *Pestalosphaeria elaeidis* is the most commonly recorded (Hyde 1996). In the phylogenetic tree, *Pestalotiopsis licualacola* clusters with *P. adusta*, *P. licualacola*, *P. rosea* and *P. trachicarpicola*. Only *P. licualacola* produces fusiform conidia, and mostly possesses a single apical appendage. The conidia of *P. licualacola* are

noticeably narrower (17.4 × 3.9 μm) than those of *P. adusta* (19 × 6 μm), *P. rosea* (19.2 × 6.2 μm) and *P. trachicarpicola* (23.5 × 6.5 μm) (Zhang *et al*. 2012, Maharachchikumbura *et al*. 2012). The apical appendages of *P. licualacola* are shorter (4–9.5 μm) than those of *P. adusta* (6–14 μm), *P. rosea* (14–22 μm) and *P. trachicarpicola* (9–18 μm) (Maharachchikumbura *et al*. 2012). In the combined dataset (ITS, beta-tubulin and *tef1* gene regions), *Pestalotiopsis* species clustered in two main subclades (A and B) with high (65% and 97%) bootstrap support (Fig. 1), which supported earlier reports (Jeewon *et al*. 2003, Liu *et al*. 2010, Maharachchikumbura *et al*. 2011, 2012). Phylogenetic analysis indicated that *P. licualacola* is a distinct species.

Hu *et al*. (2007), Liu *et al*. (2010) and Maharachchikumbura *et al*. (2012) suggested that a combined multigene dataset would better resolve the taxonomy of *Pestalotiopsis* and this approach is confirmed here. However, we can only compare our species with those with ex-type sequence data in GenBank. Several other species are known from palms but lack sequence data in GenBank. *Pestalosphaeria elaeidis* (27–34 × 6–7 μm), *Pestalotiopsis palmarum* (16–22 × 5–7 μm) and *P. macrochaeta* (22–31 × 8–10 μm) differ from *P. licualacola* (16–20 × 3–5 μm) because they have distinctly longer and wider conidia (Guba 1961, Booth & Robertson 1961, Zhang *et al*. 2003). *Pestalotiopsis phoenicis* has appendages with knobbed ends (Guba 1961, Chen & Wei 1993), while *P. gibberosa* (14–20 × 6–8 μm) differs from *P. licualacola* (16–20 × 3–5 μm) because it has wider, broadly clavate conidia with darkened central cells (Guba 1961, Chen & Wei 1993), thus confirming the novelty of *P. licualacola*.

Acknowledgments

This research was funded by Guizhou Province to tackle hard-nut problems in agricultural science and technology (No. 20113045), and the project of Guiyang Science and Technology Department [2012102]3-25. K.D. Hyde thanks the National Research Council of Thailand (grant for Pestalotiopsis No: 55201020008) for supporting this research.

References

Aly, A.H., Debbab, A., Kjer, J. & Proksch, P. (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bio-active natural products. *Fungal Diversity* 41: 1–16. http://dx.doi.org/10.1007/s13225-010-0034-4

Aly, A.H., Debbab, A. & Proksch, P. (2011) Fifty years of drug discovery from fungi. *Fungal Diversity* 50: 3–19. http://dx.doi.org/10.1007/s13225-011-0116-y

Boonmee, S., Zhang, Y., Chomnunti, P., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis. *Fungal Diversity* 51: 63–102. http://dx.doi.org/10.1007/s13225-011-0147-4

Booth, C. & Robertson, J.S. (1961) *Leptosphaeria elaeidis* sp. nov. isolated from anthracnosed tissue of oil palm seedlings. *Transactions of the British Mycological Society* 44(1): 24–26.

Chen, Y.X. & Wei, G. (1993) New combinations of *Pestalotiopsis* from China. *Journal of Guangxi Agricultural University* 12(1): 23–35.

Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., Ko-Ko, T.W., Hongsanan, S., Jones, E.B.G., Kodsueb, R., Phookamsak, R., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) *Capnodiaceae*. *Fungal Diversity* 51: 103–134. http://dx.doi.org/10.1007/s13225-011-0145-6

Debbab, A., Aly, A.H. & Proksch, P. (2011) Bioactive secondary metabolites from endophytes and associated marine derived fungi. *Fungal Diversity* 49: 1–12. http://dx.doi.org/10.1007/s13225-011-0114-0

Debbab, A., Aly, A.H. & Proksch, P. (2012) Endophytes and associated marine derived fungi—ecological and chemical perspectives. *Fungal Diversity* 57: 45–83. http://dx.doi.org/10.1007/s13225-012-0191-8

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical*

Bulletin 19: 11–15.
Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. http://dx.doi.org/10.2307/2408678
Glass, N.L. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61(4): 1323–1330. http://dx.doi.org/10.1046/j.1439-0507.2003.00837.x
Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
Hu, H.L., Jeewon, R., Zhou, D.Q., Zhou, T.X. & Hyde, K.D. (2007) Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: evidence from rDNA and β- tubulin gene phylogenies. *Fungal Diversity* 24: 1–22.
Hyde, K.D. (1996) Fungi from palms. XXV. *Pestalosphaeria elaeidis. Mycotaxon* 57: 353–357.
Jeewon, R., Liew, E.C.Y., Simpson, J.A., Hodgkiss, I.J. & Hyde, K.D. (2003) Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. *Molecular Phylogenetics and Evolution* 27: 372–383. http://dx.doi.org/10.1016/S1055-7903(03)00010-1
Kishino, H. & Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data. *Journal of Molecular Evolution* 29: 170–179. http://dx.doi.org/10.1007/BF02100115
Ko-Ko, T., Stephenson, S.L., Bahkali, A.H. & Hyde, K.D. (2011a) From morphology to molecular biology: can we use sequence data to identify fungal endophytes? *Fungal Diversity* 50: 113–120. http://dx.doi.org/10.1007/s13225-011-0130-0
Ko-Ko, T.W., McKenzie, E.H.C., Bahkali, A.H., To-Anun, C., Chukeatirote, E., Promputtha, I., Abd-Elsalam, K.A., Soytong, K., Wulandari, N.F., Sanoamuang, N., Jonglaekha, N., Kobsueb, R., Cheewangkoon, R., Wikee, S., Chamyuang, S. & Hyde, K.D. (2011b) The need for re-inventory of Thai phytopathogens. *Chiang Mai Journal of Science* 38: 1–13.
Lee, S.B. & Taylor, J.W. (1990) Isolation of DNA from fungal mycelia and single spores. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, USA, pp. 282–287.
Liu, A.R., Chen, S.C., Wu, S.Y., Xu, T., Guo, L.D., Jeewon, R. & Wei, J.G. (2010) Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in *Pestalotiopsis* taxonomy. *Molecular Phylogenetics and Evolution* 57: 528–535. http://dx.doi.org/10.1016/j.ympev.2010.07.017
Maharachchikumbura, S.S.N., Guo, L.D., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) *Pestalotiopsis*—morphology, phylogeny, biochemistry and diversity. *Fungal Diversity* 50: 167–187. http://dx.doi.org/10.1007/s13225-011-0125-x
Maharachchikumbura, S.S.N., Guo, L.D., Cai, L., Chukeatirote, E., Wu, W.P., Sun, X., Crous, P.W., Bhat, D.J., McKenzie, E.H.C., Bahkali, A.H. & Hyde, K.D. (2012) A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. *Fungal Diversity* 56: 95–129. http://dx.doi.org/10.1007/s13225-012-0198-1
O'Donnell, K. & Cigelnik, E. (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7(1): 103–116. http://dx.doi.org/10.1006/mpev.1996.0376
Rehner S (2001) Primers for elongation factor 1-α (EF1-α). Available from: http://ocid.nacas.org/research/deephyphae/EF1primer.pdf (Cited 20 August 2008).
Swofford, D.L. (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876
White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.) *PCR protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.
Xu, J., Ebada, S.S. & Proksch, P. (2010) *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. *Fungal Diversity* 44: 15–31. http://dx.doi.org/10.1007/s13225-010-0055-z
Zhang, J.X., Xu, T. & Ge, Q.X. (2003) Notes on *Pestalotiopsis* from Southern China. *Mycotaxon* 85: 91–92.