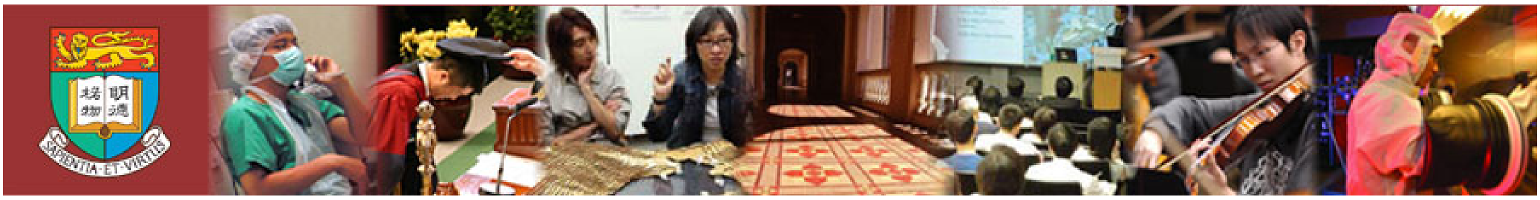

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Three new species of *Pyricularia* are isolated as zingiberaceous endophytes from Thailand

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Abstract: *Pyricularia costina* and three undescribed *Pyricularia* species were found as endophytes on wild ginger *Amomum siamense* and *Alpinia malaccensis* in Doi Suthep-Pui National Park, Chiang Mai, Thailand. Three new species, *Pyricularia kookicola, P. longispora,* and *P. variabilis* are described, illustrated and compared to similar *Pyricularia* species.
Key words: *Alpinia malaccensis, Amomum siamense,* mitosporic fungi, taxonomy

INTRODUCTION

Genus *Pyricularia* Sacc. was established by Saccardo (1880) with the single species, *P. grisea* Sacc., which commonly is found on tropical grasses. The name *Pyricularia* referred to the pyriform shape of the conidia. *Pyricularia oryzae* Cav. subsequently was described for a very similar fungus on rice (Cavara 1891), and currently there are 67 names for *Pyricularia* species in Funindex (http://194.131.255.3/cabipages/Names/NAMES.APS). Molecular studies have confirmed that *P. grisea* and *P. oryzae* are identical (Lebrun et al 1991), and the teleomorph, *Magnaporthe grisea* also has been well studied (Hebert 1971, Kato et al 1976, Yaegashi and Hebert 1976, Barr 1977). Genus *Pyricularia,* however, needs a monographic study.

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Pyricularia was circumscribed by Ellis (1971), although the distinction between it and some *Dactylaria* spp. is not always clear (Goh and Hyde 1997). The presence of a separating cell was a feature used by Ellis (1976) to distinguish *Pyricularia* from the similar genus, *Dactylaria.* The conidiogenous cells of *Pyricularia* are polyblastic, integrated on the conidiophores and are sympodial, cylindrical, geniculate and denticulate. Each denticle is cylindrical, thin-walled and cut off by a septum to form a separating cell. The conidia secede in a rhexolytic manner. They are solitary, dry, acropleurogenous, simple, obpyriform, obturbinate or obclavate, hyaline to pale olivaceous brown, and often have a protuberant hilum (Ellis 1971). In contrast, each denticle of *Dactylaria* is cylindrical, flat-topped and continuous with the conidiogenous cells. The conidia secede in a schizolytic manner and are of various shapes, usually fusiform, naviculate or cylindrical. However, in some species they may be obpyriform, obclavate, fabiform, botuliform or other shapes (Ellis 1976, Goh and Hyde 1997).

Most species of *Pyricularia* are recorded from monocotyledonous plants (Nisikado 1927, Roldan 1938, Viégas 1946, Luttrell 1954, Thirumalachar et al 1956, Rao and Reddy 1958, Veeraraghavan and Padmanabhan 1965, Prasada and Goyal 1970, 1974, Hashioka 1971, 1973, Albuquerque and Duarte 1971, Siwasin and Giatgong 1971, Rathaiah 1980, Zucconi et al 1984, Gaikwad and D'Souza 1987, Ondřej 1988, Sarbajna 1990), especially Commelinaceae, Cyperaceae, Poaceae, and Zingiberaceae. However, *Pyricularia caffera* Matsush., *P. lourinae* F.C. Albuq. & L.R. Duarte, *P. peruamazonica* Matsush., *P. subsigmoidea* R.F. Castañeda & W.B. Kendr., and *P. vandalurensis* Subram. & Vittal were described from dicotyledonous plants (Albuquerque and Duarte 1971, Subramanian and Vittal 1974, Castañeda and Kendrick 1991, Matsushima 1993, 1996). Some species originally described in *Pyricularia* subsequently have been synonymized or transferred to other genera. For example, *P. aquatica* Ingold and *P. juncicola* MacGaevie were moved to *Tumularia aquatica* (Ingold) Descals & Marvanová and *Dactylaria junci* M.B. Ellis, respectively (Ingold 1943, Ellis 1971, 1976, Marvanová and Descals 1987). In addition, *Pyricularia musae* Hughes (1958) was placed in *Pyriculariopsis* because its broad denticles are not cut off by a septum to form a separating cell, as they are in *Pyricularia* (Ellis 1971). The conidia of *Pyriculariopsis parasitica* are versicolored, with hyaline end cells and darker intermediate cells.

During our investigation of endophytic fungi on wild ginger, *Amomum siamense* Craib. and *Alpinia malaccensis* (Burm.) Rosc., at Doi Suthep-Pui National Park, Chiang Mai, Thailand, we found 41 fungal taxa, including four *Pyricularia* species, *P. costina* Sarbajna and three undescribed species. *Pyricularia* species were isolated from 8.38% of 800 healthy leaves of *Amomum siamense* sampled, and 2.12% of 800 healthy leaves of *Alpinia malaccensis* sampled (Bussaban et al 2001a, b). In this paper, the new species, *P. kookicola* Bussaban sp. nov., *P. longispora* Bussaban sp. nov. and *P. variabilis* Bussaban sp. nov. are described and compared to similar species.

MATERIALS AND METHODS

Endophytic fungi were isolated from *Amomum siamense* (1222 isolates) and *Alpinia malaccensis* (1110 isolates), using a triple sterilization method, and cultured on cornmeal agar (Bussaban et al 2001a, b). *Pyricularia* species were isolated from leaves treated by surface sterilization in 95% alcohol for 15 s, followed by 5 min in 1% sodium hypochlorite and finally, 15 s in 95% alcohol. Slides of fungi were mounted in distilled water for observation, photomicrography and measurement. Living cultures have been deposited in the National Center of Genetic Engineering and Biotechnology Culture Collection, Thailand (BCC).

TAXONOMY

Pyricularia costina Sarbajna, Journal of Mycopathological Research 28: 162. 1990. FIGS. 1, 2, 14A

Colonies on potato-dextrose agar reaching 9 cm in 10 d, effuse, grayish-brown. *Conidiophores* up to 500 μm long, 3.7–5 μm thick, usually branched, flexuous, geniculate, septate, pale brown to brown, smooth. *Conidiogenous cells* sympodial, cylindrical, geniculate, denticulate; each denticle cylindrical, thin-walled, mostly cut off by septum to form a separating cell. *Conidia* 22.5–37.5 × 7.5–10 μm, solitary, dry, obpyriform or obturbinate, hyaline, smooth, 2-septate, hilum often protuberant.

Speciments examined: THAILAND. CHIANG MAI: Doi Suthep-Pui National Park, isolated as an endophyte from leaves of *Amomum siamense*, February 2000, *B. Bussaban* CMUZE0003 (BCC8219); August 1999, *B. Bussaban* CMUZE0006; isolated as an endophyte from leaves of *Alpinia malaccensis*, August 1999, *B. Bussaban* CMUZE0141; February 2000, *B. Bussaban* CMUZE0504; CMUZE0505.

Commentary: This species has been described as a pathogen (Sarbajna 1990), causing leaf spots on *Costus speciosus* Smith (Zingiberaceae), but no spots were seen on either *Amomum siamense* or *Alpinia malaccensis*, the fungus being isolated from healthy green leaves.

Pyricularia kookicola Bussaban, sp. nov. FIGS. 3–5, 14B

Coloniae effusae, viridi-griseus. Conidiophora macronemata, usque ad 250 μm longa, 2.5–4 μm crassa, raro ramosa, flexuosa, septata, pallide brunnea vel brunnea, laevia. Cellulae conidiogenae sympodiales, cylindricis, geniculatae, denticulatae, denticulis quibusque cylindricis, tennuitunicatis cellulam intercalarem producentibus. Conidia 27–33.5 × 7.9–10 μm, solitaria, sicca, obpyriformia, hyalina, laevia, 2-septata, hilo distincte.

Colonies on potato-dextrose agar reaching 9 cm in 12 d, effuse, greenish-gray. *Conidiophores* up to 250 μm long, 2.5–4 μm thick, macronematous, rarely branched, flexuous, septate, pale brown to brown, smooth. *Conidiogenous cells* sympodial, cylindrical, geniculate, denticulate; each denticle cylindrical, thin-walled, mostly cut off by septum to form a separating cell. *Conidia* 27–33.5 × 7.9–10 μm, solitary, dry, obpyriform, hyaline, smooth, 2-septate, hilum protuberant.

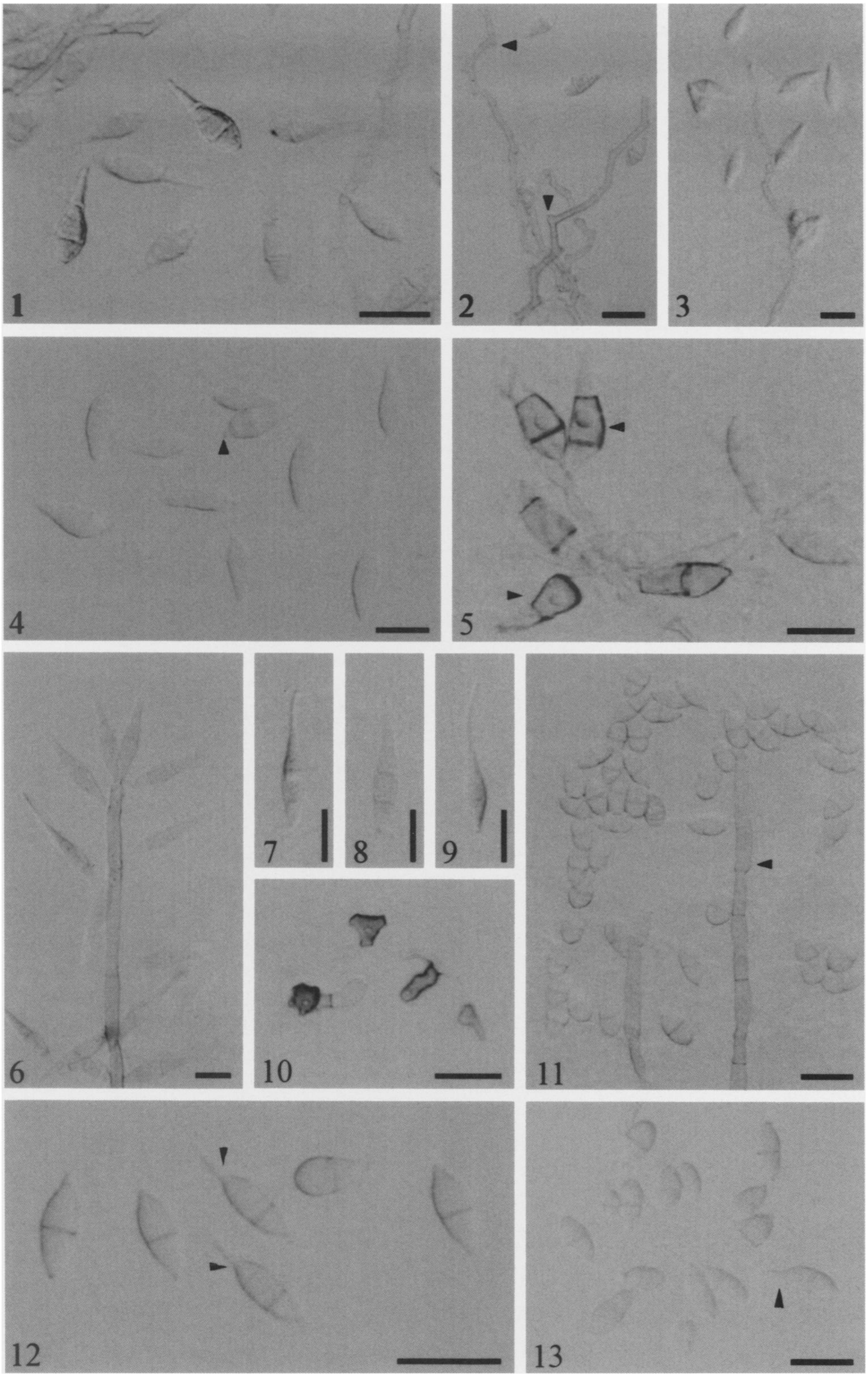
Etymology: Kook, the local northern Thai name for *Amomum siamense*, and *icola* meaning "loving".

Speciments examined: THAILAND. CHIANG MAI: Doi Suthep-Pui National Park, isolated as an endophyte from leaves of *Amomum siamense*, February 2000, *B. Bussaban* CMUZE0501 (HOLOTYPE).

Commentary: This species is similar to *Pyricularia angulata*, *P. cannaecola*, *P. distorta* and *P. zingiberi* in having 2-septate conidia. However, the conidia of *P. kookicola* are larger. *Pyricularia kookicola* also is similar to *P. guarmaicola* in conidial shape and septation, but its conidia are narrower (27–33.5 × 7.9–10 μm vs 20–28 × 10–12 μm). *Pyricularia kookicola* has similar-size 2-septate conidia as *P. caricis*, *P. costina*, *P. leersiae*, *P. penniseti* and *P. peruamazonica*. *Pyricularia kookicola* has obpyriform conidia, whereas *P. caricis* and *P. peruamazonica* have obclavate and fusiform conidia, respectively. Moreover, conidiophores of *P. caricis* mostly are nonseptate. *Pyricularia costina* are bluntly rounded at the apex, and its apical cells are more slender than those of *P. kookicola*. *Pyricularia leersiae* and *P. penniseti* differ from *P. kookicola* in that they produce subglobose to ovate appressoria. Moreover, conidia of *P. penniseti* have slender apical cells and the percentage of the length of the apical cell to the length of a conidium reaches 42–44%, in comparison to *P. kookicola*, which occupies 30–35%.

Pyricularia longispora Bussaban, sp. nov. FIGS. 6–10, 14C

Coloniae effusae, griseo-brunnae. Appressoria 12–15 μm per medius, angularis et irregularibus, stellatae, atro-brun-



FIGS. 1–13. *Pyricularia costina*, *P. kookicola*, *P. longispora* and *P. variabilis*. 1, 2. *P. costina*. 1. Conidia. 2. Conidiophores with denticles (arrowed) and conidia. 3–5. *P. kookicola* (from holotype). 3. Conidiophore and conidia. 4. Conidia with protuberant hilum (arrowed). 5. Matured conidia (arrowed). 6–10. *P. longispora* (from holotype). 6. Conidiophore and conidia. 7–9. Conidia. 10. Irregular shaped hyphopodia. 11–13. *P. variabilis* (from holotype). 11. Conidiophores with swollen intercalary nodes (arrowed) and conidia. 12, 13. One and two (arrowed) septate conidia. Scale bars = 20 μm.

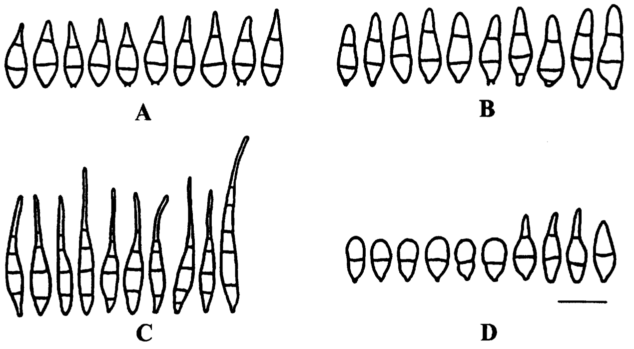


FIG. 14. *Pyricularia costina, P. kookicola, P. longispora* and *P. variabilis.* Diagrammatic representation of conidia. A. *P. costina.* B. *P. kookicola.* C. *P. longispora.* D. *P. variabilis.* Scale bars = 20 μm.

nae. Conidiophora macronemata, usque ad 400 μm longa, 3– 4.6 μm crassa, raro ramosa, recta, septata, ad basem pallide brunnea, ad apicem plerumque subhyalina. Cellulae conidiogenae cylindricis, denticulis quibusque cylindricis, tennuitunicatis cellulam intercalarem producentibus. Conidia 47–72 × 5.6–7.6 μm, solitaria, sicca, obclavata, hyalina vel pallide brunnea, laevia, 4(–5)-septata.

Colonies on potato-dextrose agar reaching 9 cm in 12 d, effuse, grayish-brown. Appressoria 12–15 μm diam, angular to irregular, stellate, dark brown. *Conidiophores* up to 400 μm long, 3–4.6 μm thick, macronematous, rarely branched, straight, septate, pale brown near the base, often subhyaline at the apex. *Conidiogenous cells* cylindrical, denticulate; each denticle cylindrical, thin-walled, mostly cut off by septum to form a separating cell. *Conidia* 47–72 × 5.6–7.6 μm, solitary, dry, obclavate, hyaline to pale brown, smooth, 4(–5)-septate.

Etymology: Referring to the length of conidia.

Speciments examined: THAILAND. CHIANG MAI: Doi Suthep-Pui National Park, isolated as an endophyte from leaves of *Amomum siamense,* February 2000, *B. Bussaban* CMUZE0005 (HOLOTYPE: BCC11377); August 1999, *B. Bussaban* CMUZE0502; isolated as an endophyte from leaves of *Alpinia malaccensis,* August 1999, *B. Bussaban* CMUZE0503.

Commentary: This species differs from other *Pyricularia* spp. in its long, 4-septate conidia. *Pyricularia angulata* (ovate-pyriform, 18.2–28 × 4.9–9.1 μm, 2-septate), *P. leersiae* (obpyriform, 27–37.5 × 6–9.7 μm, 2-septate) and *P. penniseti* (obclavate, 18.4–36.7 × 7.4–11 μm, 2-septate) also form appressoria in culture. However, conidia are very different in shape, size and septation, when compared to *P. longispora* (obclavate, 47–72 × 5.6–7.6 μm, 4(–5)-septate).

Pyricularia variabilis Bussaban, sp. nov.

FIGS. 11–13, 14D

Coloniae effusae, griseo-brunnae. Conidiophora macronemata, usque ad 700 μm longa, 3.8–5 μm crassa, septata, recta vel flexuosa, atro-brunnea, ad apicem subhyalina, laevia, ad apicem et intercalarem 5–7 μm inflata. Cellulae conidiogenae sympodiales, geniculatae, denticulatae, denticulis quibusque cylindricis, tennuitunicatis cellulam intercalarem producentibus. Conidia 15.7–28.6 × 6.4–9.3 μm, solitaria, obovatae vel obpyriformia, hyalina vel subhyalina, laevia, 1–2-septata, hilo distincte.

Colonies on potato-dextrose agar reaching 9 cm in 7 d, effuse, grayish-brown, hairy. *Conidiophores* up to 700 μm long, 3.8–5 μm thick, macronematous, septate, straight or flexuous, dark brown, subhyaline at the apex, smooth, terminal and intercalary nodes swelling 5–7 μm diam. *Conidiogenous cells* sympodial, cylindrical, geniculate, denticulate; each denticle cylindrical, thin-walled, mostly cut off by septum to form a separating cell. *Conidia* 15.7–28.6 × 6.4–9.3 μm, solitary on denticles arising from terminal and intercalary swellings, obovoid or obpyriform, hyaline to subhyaline, smooth, 1–2-septate, hilum protuberant.

Etymology: Referring to variation of shape of conidia.

Speciments examined: THAILAND. CHIANG MAI: Doi Suthep-Pui National Park, isolated as an endophyte from leaves of *Amomum siamense,* February 2000, *B. Bussaban* CMUZE0229 (HOLOTYPE: BCC8210); August 1999, *B. Bussaban* CMUZE0506.

Commentary: Pyricularia variabilis differs from other species in the genus in its variable conidial shape. It also has swollen, terminal and intercalary nodes on the conidiophores, a feature not found in other species of *Pyricularia.*

Five species of *Pyricularia* have been found to be parasitic on Zingiberaceae. Nisikado (1927) isolated *P. zingiberi* Nisik. from common ginger (*Zingiber officinale* Rosc.) and Japanese wild ginger (*Z. mioga* Rosc.). Hashioka (1971) described a new species, *P. distorta* Hashioka, as a blast fungus on *Catymbium* sp. and *Alpinia* sp. in Thailand, while Siwasin and Gaitgong (1971) reported *P. globbae* Siwasin & Giatgong, infecting *Globba* sp. in Thailand. In India, *P. costina* Sarbajna and *P. curcumae* Rathaiah were reported as pathogens, causing leaf spots of *Costus speciosus* Smith and leaf blast of turmeric, *Curcuma longa* L., respectively (Rathaiah 1980, Sabajna 1990). This study is the first report of *Pyricularia costina* and three new *Pyricularia* spp. as endophytes of wild ginger.

Some endophytes are thought to benefit host plants by protecting them against insect pests and plant pathogens, conferring drought tolerance, or enhancing absorption of soil nutrients (Webber 1981, Funk et al 1983, Carroll 1986, 1988, Thomson et al 1986, Clay 1989, Breen 1993, 1994, Stone et al 2000). Endophytic fungi may develop as saprobes, once a plant senesces or a leaf dies. However, they also might be latent pathogens and develop to cause plant diseases under some conditions (Latch 1993, Brown et al 1998). Photita et al (2001) found *Pyriculariopsis parasitica* to be a common endophyte of *Musa acuminata* Colla, but it also is pathogenic on bananas (Meredith 1962, Stover 1972). In *Amomum siamense* and *Alpinia malaccensis*, *Pyricularia* species were isolated from leaves, not from pseudostems or rhizomes (Bussaban et al 2001a, b). They might be pathogenic or saprobic, and it is unknown what role endophytic species isolated from ginger in Thailand might play.

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