

Two new *Curvularia* species from northern Thailand

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A multi-locus phylogeny based on combined sequences of rDNA ITS, EF1- α , GPDH and LSU revealed two new saprobic *Curvularia* species from grasses and dead wood in northern Thailand. The novel species, *Curvularia alcornii* and *C. asianensis* are introduced, fully described, illustrated, and compared to similar taxa in the genus.

Keywords: pathogen, phylogeny, taxonomy, saprobe.

Species of *Curvularia* Boedjin (1933) are pathogens, saprobes and endophytes mostly on grasses, but they can also be found on other hosts. Manamgoda *et al.* (2012) established a multi-locus phylogeny for the *Bipolaris*/*Cochliobolus*/*Curvularia* complex, showing this group to comprise two major clades. They maintained *Bipolaris* and *Curvularia* for these clades and amended the genera based on new phylogenetic data and conidial characteristics. Nine *Bipolaris* species were transferred to *Curvularia* as a result of this study. Conidia of *Curvularia* tend to be shorter than those of *Bipolaris* (in most species less than 100 μm) and often curved. The conidia often have intermediate cells which are inordinately enlarged and results in their characteristic curvature. The conidia of *Bipolaris* species are usually larger and have more septa than those of *Curvularia* and can be straight or gently curved. Stromata may form below the ascoma body in the sexual state of *Curvularia*; such formation is not found in *Bipolaris*.

Accurate species identification in *Curvularia* is difficult. Many species have vague descriptions without illustrations (Hosokawa *et al.* 2003). Conidial characters often vary in colour and degree of curvature and this may depend on environmental conditions, host, substrate and media (Upsher 1975; Tsuda & Ueyama 1982, 1983; Tsuda 1992; Hosokawa *et al.* 2003). With the application of molecular methods, cryptic species which cannot be distinguished by morphological characters are resolved (Cai *et al.* 2011); thus it

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is essential to use molecular techniques in species identification of *Curvularia*. In the present study we carried out a phylogenetic analysis of 23 isolates of *Curvularia* and 13 *Curvularia* type cultures using rDNA ITS (internal transcribed spacer), GPDH (glyceraldehyde 3-phosphate dehydrogenase), LSU (large subunit of nuclear ribosomal DNA) and EF1- α (translation elongation factor 1- α). Two new species of *Curvularia* were revealed from the combined data analyses and are introduced, described and illustrated.

Materials and methods

Isolation and morphological studies

Plant-pathogenic and saprobic strains of *Curvularia* were collected in field surveys from various hosts in Chiang Rai and Chiang Mai Provinces of northern Thailand (Tab. 1). Fresh specimens were incubated for 24 to 48 hours in a moist chamber before isolation. Specimens were observed under a stereo microscope and conidia were taken from the sporulating samples for single-spore isolation by a modified spore suspension method as described for different fungal groups (Choi *et al.* 1999, Chomnunti *et al.* 2011, Liu *et al.* 2011). Conidia were placed in 400 μ l sterilized water on a sterilized glass slide. The spore suspension was then transferred to water agar (WA) plates using a sterilized pipette tip. The WA plates were incubated overnight (12 h) to allow spores to germinate and germinated spores were then individually transferred to PDA. All fresh cultures and herbarium material were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and MFLU herbarium, respectively. Duplicate cultures were deposited in BCC. Details of nomenclatural novelties were added to MycoBank (Crous *et al.* 2004). Details of all ex-type strains and fresh strains used are listed in Tab. 1.

Morphological observations

Morphological characters of species including conidial length, width, conidial septation, conidiophore length and width and attachment of conidia to the conidiophores were determined from the host substrate and on PDA media using a compound light microscope (Nikon Eclipse 80i). A slide culture technique was used to observe conidiophores and attachment of conidia (Riddell *et al.* 1950, Su *et al.* 2012). More than 30 conidia of each isolate were measured. Conidial colour was compared using colour charts (Rayner 1970). Three duplicate cultures of each isolate were used for determining colony characters on potato-dextrose agar (PDA, Difco) at 25 °C in the dark. Growth rate, colony colour and zonation were recorded from three replicates of 7 day-old cultures grown on PDA at 25 °C.

DNA extraction, PCR and phylogenetic analysis

DNA was extracted from the fresh mycelium, PCR amplification and sequencing were conducted as described in Manamgoda *et al.* (2012). Sequences of ITS, GPDH, EF1- α and LSU gene regions were combined and the

Tab. 1. Details of isolates subjected to multigene DNA sequence analysis; ex type cultures and sequences are bold.

| Species | Accession No. | Host | Location | GenBank No. | | | | Reference |
|---------------------------------|-----------------------|------------------------------|-------------|-----------------|-----------------|-----------------|-----------------|---|
| | | | | ITS | GPDH | LSU | EF1- α | |
| <i>Bipolaris chloridis</i> | CBS 242.77 | <i>Chloris gayana</i> | Australia | JN192372 | JN600961 | – | – | Manamgoda <i>et al.</i> 2011 |
| | MFLUCC 10-0703 | <i>Zea mays</i> | Thailand | JX256420 | JX276433 | JX256387 | JX266589 | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia astanensis</i> | MFLUCC 10-0705 | <i>Panicum sp.</i> | Thailand | JX256421 | JX276434 | JX256388 | JX266590 | Manamgoda <i>et al.</i> 2012 |
| | MFLUCC 10-0687 | <i>Oryza sativa</i> | Thailand | JX256422 | JX276435 | JX256389 | JX266591 | Manamgoda <i>et al.</i> 2012 |
| | MFLUCC 10-0704 | Bamboo | Thailand | JX256423 | – | JX256390 | JX266592 | Manamgoda <i>et al.</i> 2012 |
| | MFLUCC 10-0711 | <i>Panicum sp.</i> | Thailand | JX256424 | JX276436 | JX256391 | JX266593 | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia australiensis</i> | MFLUCC 10-0685 | <i>Saccharum officinarum</i> | Thailand | JX256425 | JX276437 | JX256392 | JX266594 | Manamgoda <i>et al.</i> 2012 |
| | CBS 172.57 | <i>Oryza sativa</i> | Vietnam | JN61026 | JN61036 | JN600981 | JN601003 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia coicis</i> | CBS 192.29 | <i>Coix lacryma-jobi</i> | Japan | AF081447 | AF081410 | JN600984 | JN601006 | Manamgoda <i>et al.</i> 2011 Berbee <i>et al.</i> 1999 |
| <i>Curvularia ellisii</i> | CBS 193.62 | <i>Air</i> | Pakistan | JN192375 | JN600963 | JN600985 | JN601007 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia gladioli</i> | ICMP 6160 | <i>Gladiolus sp.</i> | New Zealand | JX256426 | JX276438 | JX256393 | JX266595 | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia graminicola</i> | BRIP 23186 | | Australia | JN192376 | JN600964 | JN600986 | JN601008 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia hawaiiensis</i> | BRIP 15933 | <i>Chloris gayana</i> | Australia | JN601028 | JN600965 | JN600987 | JN601009 | Manamgoda <i>et al.</i> 2011 |

| Species | Accession No. | Host | Location | GenBank No. | | | | Reference |
|---------------------------------|-------------------|------------------------------|-------------|-----------------|-----------------|-----------------|-----------------|------------------------------|
| | | | | ITS | GPDH | LSU | EF1- α | |
| <i>Curvularia heteropogonis</i> | CBS 284.91 | <i>Heteropogon contortus</i> | Australia | JN192379 | JN600969 | JN600990 | JN601013 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia ischaemi</i> | ICMP 6172 | <i>Ischaemum indicum</i> | New Zealand | JX256428 | JX276440 | JX256395 | - | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia lunata</i> | CBS 730.96 | human lung biopsy | USA | JX256429 | JX276441 | JX256396 | JX266596 | Manamgoda <i>et al.</i> 2012 |
| | CBS 157.34 | Unknown | Indonesia | JX256430 | JX276442 | JX256397 | JX266597 | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia ovariicola</i> | CBS 470.90 | <i>Eragrostis interrupta</i> | Australia | JN192384 | JN600976 | JN600998 | JN601020 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia perotidis</i> | CBS 350.90 | <i>Perotis rara</i> | Australia | JN192385 | - | JN600999 | JN601021 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia ravenelii</i> | BRIP 13165 | <i>Sporobolus fertilis</i> | Australia | JN192386 | JN600978 | JN601001 | JN601024 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia spiciifera</i> | CBS 274.52 | Soil | Spain | JN192387 | JN600979 | JX256400 | JN601023 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia tripogonis</i> | BRIP 12375 | Unknown | Australia | JN192388 | JN600980 | JN601002 | JN601025 | Manamgoda <i>et al.</i> 2011 |
| <i>Curvularia tuberculata</i> | CBS 146.63 | <i>Zea mays</i> | Rajasthan | JX256433 | JX276445 | JX256401 | JX266599 | Manamgoda <i>et al.</i> 2012 |
| <i>Curvularia trifolii</i> | ICMP 6149 | <i>Setaria glauca</i> | New Zealand | JX256434 | JX276457 | JX256402 | JX266600 | Manamgoda <i>et al.</i> 2012 |

sequences were aligned with Clustal X (Thompson *et al.* 1997) and optimized by the online sequence alignment tool MAFFT (Katoh *et al.* 2009). Parsimony analyses were performed in PAUP v4.0b10 (Swofford 2002) to obtain phylogenetic trees. Trees were inferred using the heuristic search option with 1000 random sequence additions. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Related Consistency Index [RC] and Homoplasy Index [HI]) were calculated. Trees were figured in Treeview (Page 1996). We analyzed the newly generated sequences with all available type-derived sequences listed in Manamgoda *et al.* (2011, 2012); a sub-set of taxa was selected to infer the combined phylogenetic tree presented here (Fig 1).

Results

Phylogenetic analysis of combined ITS, GPDH, LSU and EF1- α

The combined data matrix contains 24 taxa including the outgroup. The statistics for the parsimony analysis revealed that from the 2674 characters, 2390 characters are constant (135 characters are excluded), 149 characters are parsimony-informative, while 135 variable characters are parsimony-uninformative. The best tree resulting from the parsimony analysis of the combined dataset is presented here (Fig. 1) (TL = 507, CI = 0.651, RI = 0.607, RC = 0.395, HI = 0.332). Two distinct species were resolved based on phylogeny coupled with morphological characters and are described below.

Taxonomy

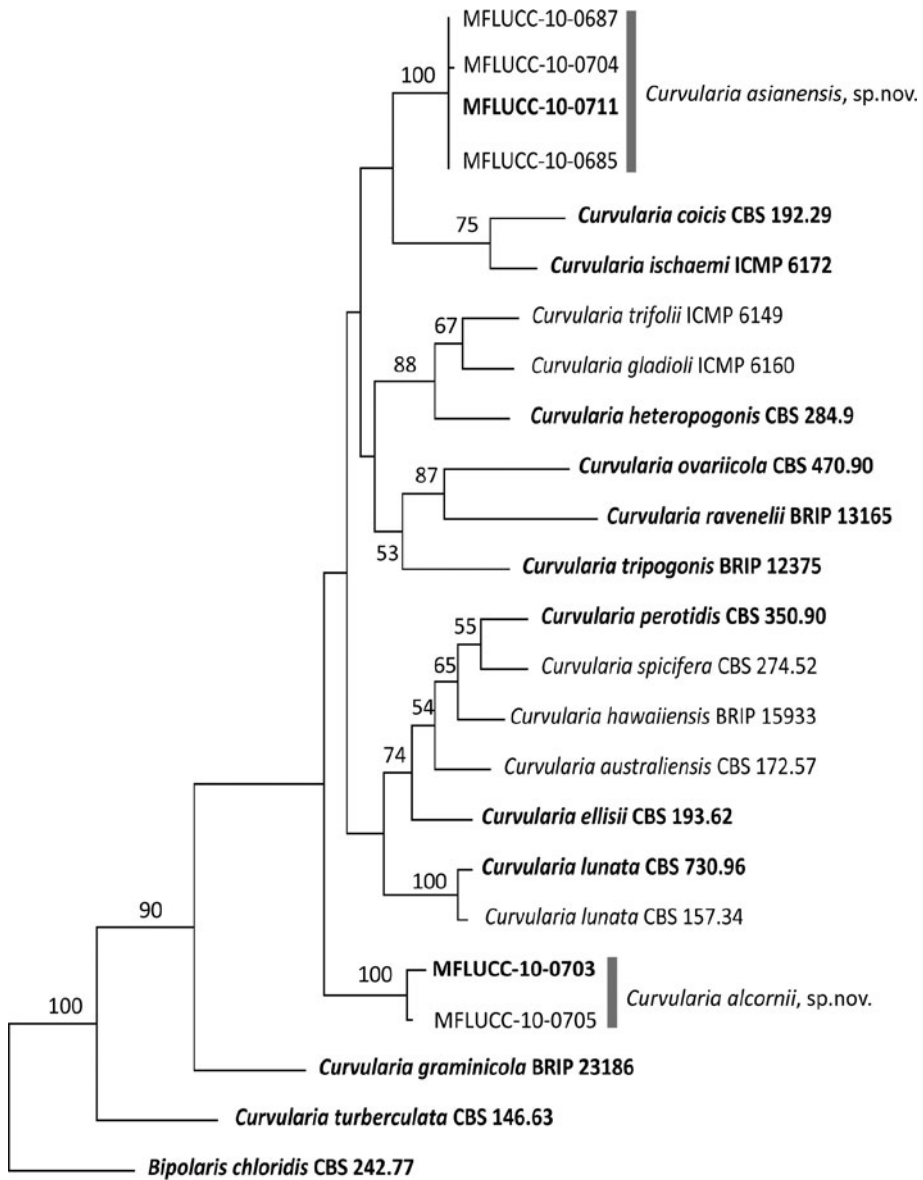
Curvularia alcornii Manamgoda, L. Cai & K. D., Hyde, **sp. nov.** – Fig. 2
MycoBank: MB 800665

Holotype. – MFLU 12-0397.

Description. – Colonies on PDA. Conidiophores (25)30–300(305) μm long, simple or branched, septate, sometimes geniculate at the apex, rust (39) to chestnut (40) coloured, with integrated conidiogenous cells, 2–3 μm wide at the base and widening to 5–7 μm at the apex. Conidia usually straight, rarely slightly curved, inequilateral, ellipsoidal or clavate, (19)21–26 (26.7) \times (8.2)9–11(12) μm ($n = 32$), 3–4-distoseptate, third cell from the base usually larger than the other cells, rust (39) to chestnut (40) coloured when mature, apical and basal cells hyaline or slightly brown, with a distinctly protuberant basal hilum. Colonies slightly convex, velvety, whitish and becoming pale olivaceous grey (120) when mature, growing slowly, reaching 4–5 cm diam. within 10 days at 25 °C in the dark. After 3–4 weeks colonies form black thickened hypae (Fig. 2 b) up to 2–3 cm long. Mycelium on host superficial, hyphae hyaline, septate, smooth-walled and 2–3 μm wide.

Etymology. – A tribute to J. L. Alcorn for his work on the generic complex.

Habitat and host. – Found as a saprobe on *Zea mays* and *Pennisetum clandestinum*.



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Fig. 1. Phylogram of *Curvularia* spp. generated from parsimony analysis based on combined genes of ITS, GPDH, LSU and EF1- α sequence data derived from ex-type and isolates from northern Thailand. The tree is rooted with *Bipolaris chloridis* CBS 242.77. Bootstrap values of more than 50 are shown in the tree. The two new species are highlighted; ex-type cultures are in bold.

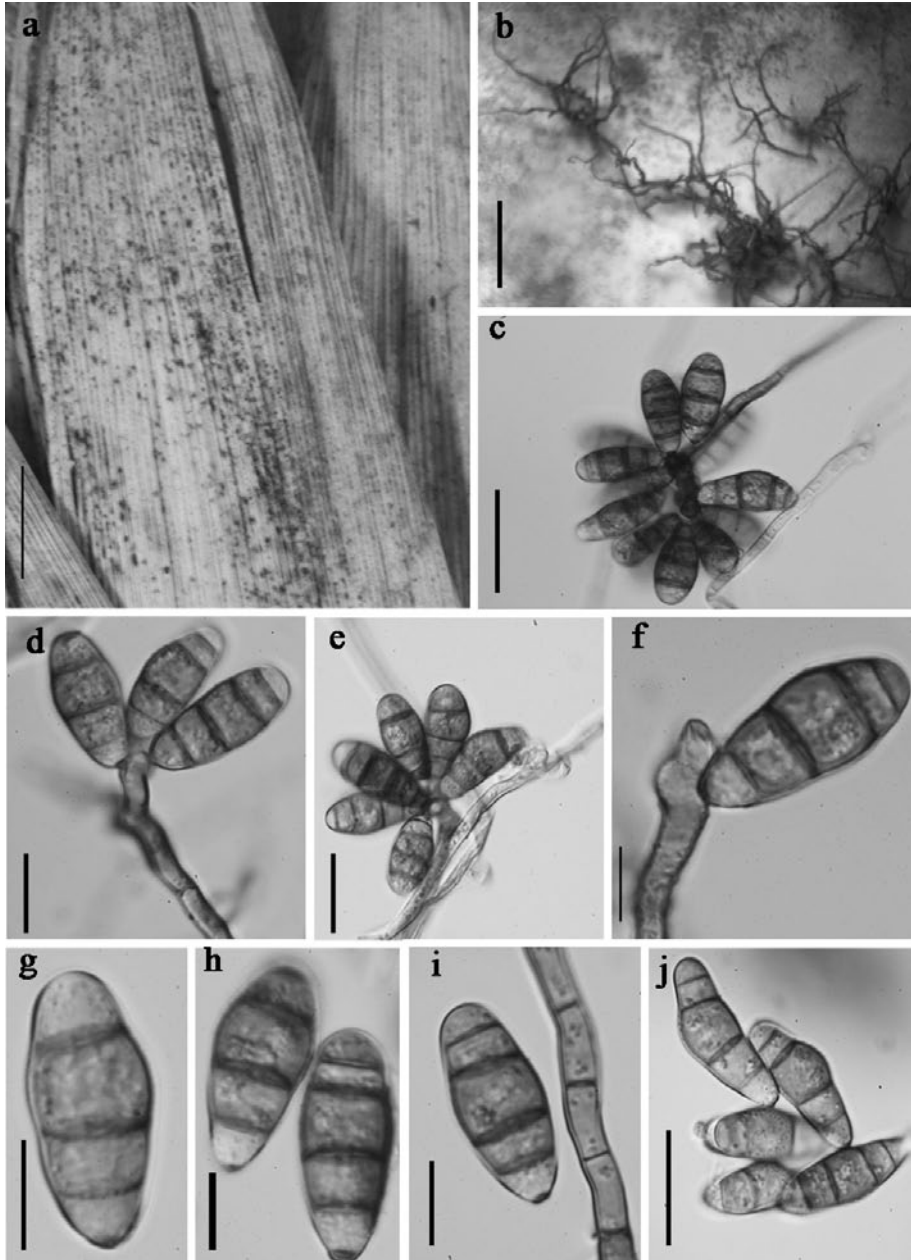


Fig. 2. Morphology of *Curvularia alcornii* (holotype). **a** Colonies on a dried husk of *Zea mays*; **b** Thickened hyphae produced on PDA; **c-f** Conidia attached to conidiophores; **h-j** conidia; bars: **a** 5 cm, **b** 1 cm, **c** 35 μ m, **d, e, j** 20 μ m, **f** 15 μ m, **g, h, i** 10 μ m.

Distribution. – Northern Thailand.

Material examined. – THAILAND, Chiang Rai Province, Muang District, Thasud Sub district, roadside in front of Mae Fah Luang University, N 18° 05' 59", E 102° 40' 02", elevation 480 m, 15 May 2010, saprobic on *Zea mays*, D. S. Manamgoda MDM0047 (MFLU12-0397, holotype), culture = MFLUCC 10-0703; *ibid.*, on dead leaf of *Pennisetum clandestinum*, 27 Apr 2010, D. S. Manamgoda MFU0048 (MFLU12-0398 isotype), culture = MFLUCC 10-0705.

Other material examined. – *Curvularia lunata* (Wakker) Boedijn: USA on *Hordeum vulgare* A 16M40 (BPI 626381 holotype); USA, Human lung biopsy (CBS 730.96 neotype).

Notes. – *Curvularia alcornii* has conidial dimensions similar to *C. lunata* but differs phylogenetically from the neotype of *C. lunata* CBS 730.96 (Fig. 1). Formation of stromatal hyphae is a character of *C. lunata* var. *aeria* (Bat., J.A. Lima & C.T. Vascon.) M.B. Ellis which produces large, black, simple or branched stromatal hyphae abundantly on rice grains (Sivanesan 1987). Because of the morphological differences between *C. lunata* and *C. lunata* var. *aeria*, Nakada *et al.* (1994) suggested that they were separate species. We compared the ITS sequences of the ex-type strain of *Curvularia lunata* var. *aeria* (CBS 294.61) with that of *C. alcornii*. *Curvularia lunata* var. *aeria* does not cluster with *C. alcornii*. *Curvularia lunata* var. *aeria* is not included in the combined phylogenetic tree as only the ITS sequence was available for this species. As *C. alcornii* is phylogenetically different from the type of *C. lunata* and *C. lunata* var. *aeria* we propose it as a new species.

Based on a blast search of NCBI's GenBank nucleotide database, the closest matches for the ITS sequence of *C. alcornii* were two strains identified as an undescribed *Curvularia* species (IPL2) isolated from *Ipomoea carnea* in India (JQ765410; identities = 477/477, 100 %) and an endophytic Dothideomycete sp. (mx125) isolated from the sapwood of *Hevea brasiliensis* (JQ905818; identities = 477/477, 100 %)

Curvularia asianensis Manamgoda, L. Cai & K. D. Hyde, **sp. nov.** – Fig. 3
Mycobank: MB 800646

Holotype. – MFLU12-0393.

Description. – Colonies on PDA. Conidiophores highly variable in length, (75–)100–700(–708) μm , simple or branched, septate, sometimes geniculate at the apex, with terminal, integrated conidiogenous cells, 2–3 μm wide at the base and widening to 4–5 μm at the apex. Conidia straight or slightly curved, very rarely strongly curved, ellipsoidal, (11)15–23(23.6) \times (6.1) 8–12(13.2) μm (n = 31) 3–4 distoseptate, apical cell hyaline, other cells umber (9) to rust (39) coloured, with a distinctly protuberant basal hilum, sometimes producing 4-septate Y-shaped conidia. The conidia on the host differed from those in culture by having a hyaline apical cell with warty ornamentation (Fig. 3 b). Colonies convex, velvety, pale olivaceous grey to olivaceous grey (120, 121), growing sparsely, reaching 5–6 cm diam. within five days at 25 °C in dark. Mycelium on host superficial, hyphae hyaline, septate, smooth-walled and 2–3 μm wide.

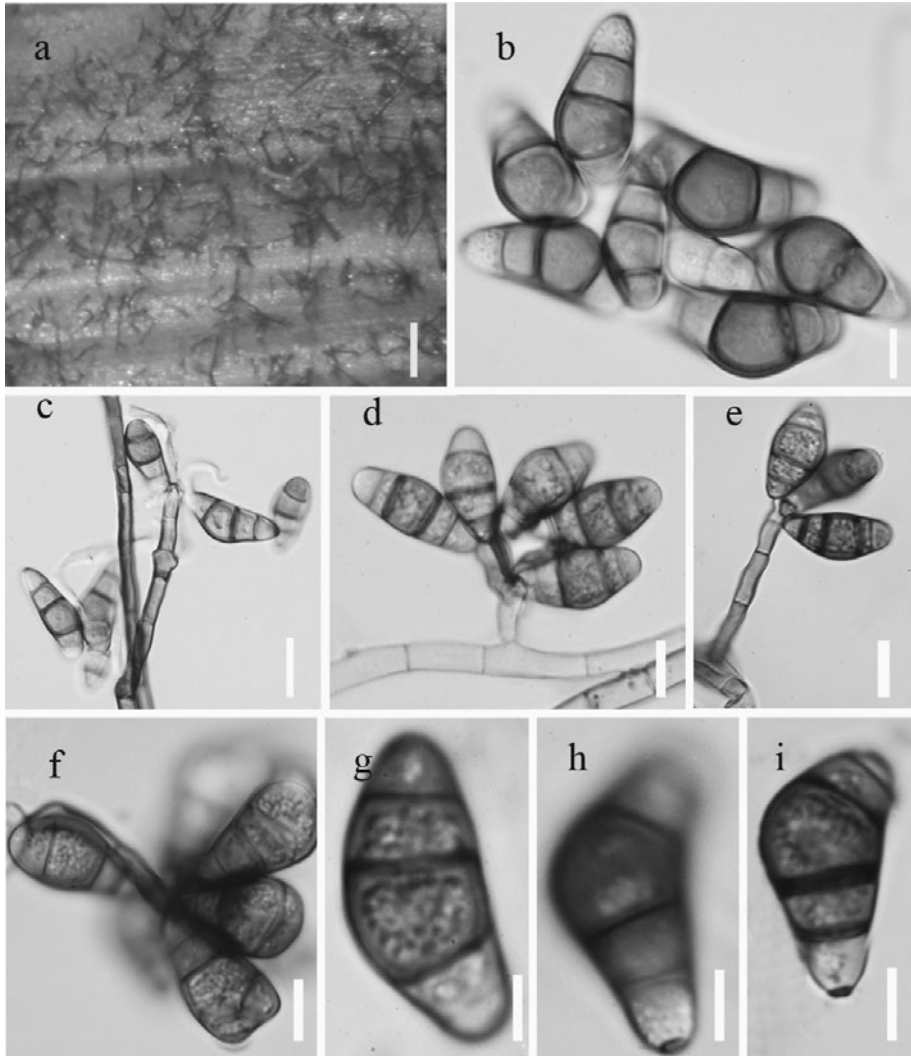


Fig. 3. Morphology of *Curvularia asianensis* (holotype). **a** Conidia and conidiophores produced on *Panicum* sp. leaf; **b** conidia produced on host; **c** germinating conidia on host; **d, e** conidiophores and conidia produced on PDA; **f** an Y shaped conidium attached to conidiophores found on PDA; **g–i** conidia produced on PDA; bars: **a** 200 μm , **b, g–i** 5 μm ; **c–f** 10 μm .

Etymology. – *asianensis* in reference to the continent where the species was found.

Habitat and host. – Saprobic on *Panicum* spp., leaves of *Saccharum officinarum* (sugarcane), grains of *Oryza sativa* (rice).

Distribution. – Northern Thailand.

Material examined. – THAILAND, Chiang Rai Province, Muang district, Thasud subdistrict, Mae Fah Luang University Park, N 18° 05' 59.1", E 102° 40' 02.9", elevation

488 m, 25 May 2010, on dried *Panicum* sp. leaf, D. S. Manamgoda MFU0058 (MFLU12-0393 holotype), culture = MFLUCC 10-0711; *ibid.*, on dead wood, 8 Jun 2010, Dhanushka Udayanga MDN0050 (MFLU12-0395), culture = MFLUCC 10-0704; THAILAND, Chiang Rai Province, Muang district, Thasud subdistrict, Roadside paddy field 20° 02' 36.51" N 99° 53' 35.98" E, elevation 572 m, on seed of *Oryza sativa*, 25 May 2010, D. S. Manamgoda MCM0025 (MFLU12-0394 isotype), culture = MFLUCC-10-0687; Chiang Rai Province, Muang district, Thasud subdistrict, on *Saccharum officinarum*, 7 May 2010 N. F. Wulandari MNL0023 (MFLU12-0396), culture = MFLUCC 10-0685 THAILAND, Tambon Huay Chompoo, Muang District, paddy field near Khun Korn Waterfall natural park, N 19° 51-54', E 99° 35'.39", elevation 1208 m, on seed of *Oryza sativa*, 28 April 2010, D. S. Manamgoda MKH0030 (living culture: MFLUCC 10-0717).

Other material examined. – *Curvularia geniculata* (Tracy & Earle) Boedijn cultured on barley seeds embedded in Sachs agar, R. R. Nelson (BPI 626383).

Notes. – The species was collected as a saprobe on three grass hosts on different occasions. Based on a blast search of GenBank nucleotide database, the closest matches for the ITS sequence of *C. asianensis* were *C. geniculata* isolated from sugar cane (JQ783058; identities = 505/505, 100%), *C. fallax* Boedijn (HNHY001) isolated from rice (JQ360963; identities = 505/505, 100%) and *C. affinis* Boedijn (ZXL07096A1) isolated from *Setaria viridis* (GU073105; identities = 505/505, 100%).

The isolates giving the best blast matches are not type cultures and may be wrongly named. However the conidial length × width measurements of *C. fallax* and *C. affinis* are (24)30.6(38) × (10)12.2(16) µm and (27)32(49) × (8)10(13) µm, respectively, and longer than those of *C. asiatica*. *Curvularia geniculata* shows a greater conidial length range in culture (18–37 µm). *Curvularia asiatica* produces longer conidiophores than *C. geniculata*. Also *C. geniculata* usually produces distinctly curved conidia whereas *C. asiatica* produces both curved and straight conidia. Conidia of *C. geniculata*, *C. fallax* and *C. affinis* are almost always reported as 4-septate, but in *C. asiatica* they are always 3- and 4-septate in culture and on the host. Based on these data it is concluded that *C. asianensis* is a new species.

Discussion

Manamgoda *et al.* (2011, 2012) showed that ITS, GPDH, EF1- α , LSU are useful markers in species delimitation within the genus *Curvularia* and the sister genus *Bipolaris*. There is a need for taxonomic assessment of this *Curvularia* based on worldwide collections. Identification of the two new species as saprobes based on grass and wood hosts shows that there is a high diversity of saprobic *Curvularia* species in the tropics.

Curvularia alcornii and *C. asianensis* may have a wider host range. Many saprobic *Curvularia* species have been found in association with a wide range of hosts (Sivanesan *et al.* 1987, Berbee *et al.* 1999, Manamgoda *et al.* 2011) and they sometimes also occur as pathogens and endophytes. Morphological species identification in the genus *Curvularia* is challenging as the conidia and conidiophore dimensions often overlap.

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