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The Merosporangiferous Fungi from Taiwan (VIII): Two New Records of *Coemansia* (Kickxellales, Kickxellomycotina)

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ABSTRACT: During an investigation of Taiwan Zygomycetes, *Coemansia asiatica* and *C. pectinata* (Kickxellaceae, Kickxellales, Kickxellomycotina) were isolated respectively from dung and soil sources and are reported as new records from Taiwan. Identification was based on morphological characters and ribosomal DNA sequence data. In this paper these two species are described, illustrated and compared with their related species.

KEY WORDS: *Coemansia*, Kickxellales, Kickxellomycotina, ribosomal DNA, Taiwan, Zygomycetes.

INTRODUCTION

The genus *Coemansia* Van Tighem & Le Monnier is the largest group in Kickxellales R. K. Benj. (Kickxellomycotina) and is known to consist of 20 species (Hibbett et al., 2007; Kirk et al., 2008). Members of this genus are small saprobes, which can be isolated from forest soil, pastureland, plant root, leaf litter and faeces of animals, such as mouse, pig, duck and frog (Linder, 1943; Chien, 1971; Kwaśna et al., 1999; Benny et al., 2001; Kurihara et al., 2000, 2008; Ho and Hsu, 2005). Some species were isolated from specific sources such as carcass of horses and died coleopterous larvae (Linder, 1943; Kwaśna et al., 1999). They are rarely encountered from the nature, and many species have never been rediscovered or cultured successfully since their original descriptions. That might be attributed to their different nutritional preferences and optimal temperature conditions (Kwaśna et al., 1999; Kurihara et al., 2008).

The typical characters of *Coemansia* spp. are yellowish colony color, septa of vegetative hyphae and sporangiophores possessing central pore with median biconvex plug. The sporangiophores are erect, simple or branched. The upper portion of sporangiophore is the fertile region bearing a number of sporocladia. The fertile regions are straight or spirally twisted. The septate, boat shaped sporocladia arising from a stalk bear plural subspherical pseudophialides excluding the sterile terminal cell on the tip. The pseudophialides are sporogenous cells and produce acrogenously unispored sporangiola. The sporangiospores are closely approximal to the wall of sporangiola and are fusiform or elliptical with taper apex and truncate base. The zygospores are nearly globose and the sexual hyphae are similar to the vegetative hyphae (Benjamin, 1979). Up to date, three species of *Coemansia*, *C. aciculifera* Linder, *C. furcata* Kurihara et al. and *C. interrupta* Linder have been reported in Taiwan (Ho and Hsu, 2005; Kurihara et al., 2000). In this study, we add two more new records, *C. asiatica* Kurihara & Sukarno and *C. pectinata* Bainier isolated from herbivore excrement and soil respectively.

MATERIALS AND METHODS

Sample collection and observation

Sample collection, isolation and purification followed Chuang and Ho (2009), except Peptone-Yeast Extract-Dextrose agar (PYED: 1g peptone, DIFCO; 1 g of yeast extract, BD; 0.5 g of dextrose, SIGMA; 15 g of agar, BIONOVAS; 1L of distilled water; adjust pH to 6.5) were also used for purification. Characteristic observation and photographing were made under light and scanning electron microscopy as described previously (Hsu and Ho, 2010). Identification was mainly based on keys of Linder (1943) and Benny (2005, unpublished).

DNA extraction and analysis

The harvested mycelium of *Coemansia* strains were prepared by culturing in 1/4 Malt Extract- Yeast Extract (ME-YE: 3 g of malt extract, DIFCO; 3 g of yeast extration, BD; 5 g of peptone, DIFCO; 10 g of dextrose, SIGMA; 1L of distilled water) liquid medium for 2 weeks. DNA extraction were performed with CTAB-mini DNA extraction method (Graham et al. 1994).

The sequences of LSU, SSU and ITS rRNA genes were amplified with primer pairs, NL1 + NL4, PNS1

+NS8Z, and ITS5 + ITS4 respectively (O'Donnell et al. 1998; White et al. 1990). The amplifing mixture contained 25 μl of 2× Taq master Mix Red (0.4mM dNTPs, 4.0 mM $MgCl_2$ and 0.05 units/μL Ampliqon *Taq* DNA polymerase, Ampliqon), 0.5 μM each primer, 1μl of extracted DNA template (about 50 ng) and ddH_2O to total 50μl final volume. Thermal cycling parameters included an initial denaturation at 94ºC for 2 min, followed by 40 cycles consisting of denaturation at 94ºC for 30 sec, annealing at 53ºC or 56ºC for 1 min, and extension at 72ºC for 1-2 min, and a final extension at 72ºC for 15 min. Polymerse chain reactions were carried out by using a PCR machine (GeneAmp® PCR System 9700, Applied Biosystems). The size of the PCR products was confirmed and concentrated by electrophoresis on a 1% agarose gel and purified using a Gel Elution Kit (GeneMark). The sequencing of the PCR products was performed using an automated DNA sequencer (Applied Biosystems 3730xl DNA Analyzer, ABI). The alignment and computation of sequences were performed by BioEdit software (Hall, 1999).

TAXONOMIC TREATMENTS

1. ***Coemansia asiatica*** Kurihara & Sukarno., Mycoscience 49: 250-257. 2008. Fig. 1

Colonies on PYED yellow. Sporangiophores septate, erect, arising from media, simple or furcate, occasionally trifurcate, upper portion fertile, producing sporocladia laterally, 12-15 μm diam, up to 1500 μm high. Sporocladia septate, boat shaped, 22-38 × 5-7 μm, composed of 6-8 cells, terminal cell sterile, recurve and rounded apically, with one-celled stalk (5-8 × 3-5 μm), racemosely arranged on the fertile portion, two to five on each sporangiophore cell segment (50-70× 7-10 μm/cell). Pseudophialides plurally arising from a sporocladium, flask-shaped, 4-6 × 1.5-2 μm, acrogenously forming a sporangiolum. Sporangiola monosporic, hyaline, elliptical, fusiform, 11-13 × 2-3 μm (length / wide = 4.8), rounded distally and truncate below; distal sporangial wall distinct from the apex of the sporangiospore inside. Sporangiospores fusiform, slightly tapering towards the apex, 10-12.5 × 2-3 μm, circumambient within a sporangiolum. Zygospores not observed.

Specimens examined: *TDDCOW* = BCRC 34628, isolated from Water Buffalo (*Bubalus bubalis*) excrement, Taitung County, Taiwan, July 2009, coll. Y.T. Chen, isol. S.C. Chuang.

Note: Morphologically, *C. asiatica* resembles *C. mojavensis* R.K. Benjamin in having dichotomously branched sporangiophores, sporocladia densely arranged on sporangiophores, and producing slightly curved sporangiospores. However, the spore apex of *C. mojavensis* is more pointed than those of *C. asiatica* (Benjamin, 1958; Kurihara et al., 2008). The sporangiospores of *TDDCOW* are only slightly tapering towards the apex, we thus identified *TDDCOW* as *C. asiatica. TDDCOW* grew and sporulated well on PYED and 1/2 ME-YE media, but did not grow on CMA. *Coemansia* species often produce delicate fruity aromas when they are grown on 1/2 ME-YE and other agar media (Kurihara et al. 2008). When cultured on PYED , *TDDCOW* produced narcissus-like fragrance as Japan isolate (*C. asiatica,* NBRC 102546) did on 1/2 ME-YE. The sporangiola of *TDDCOW* (2-2.5 μm) are thinner than those of NBRC 102546 (2.5-3.5 μm) (Kurihara et al., 2008).

In addition to the morphological characters, the molecular data also supported our identification. There was only one difference (one substitution in 681 bp) within the partial sequences of LSU rDNA between *TDDCOW* and NBRC 102546, and one difference (1 substitution in 2834 bp) within the partial sequences of SSU rDNA, although *TDDCOW* and BTCC-F31 (ex-type strain of *C. asiatica*) showed slight differences in the ITS rDNA region (10 differences including four substitutions and six indels of 952 bp (Table 1).

2. ***Coemansia pectinata*** Bainier, Bull. Soc. Myc. France 22: 216-218. 1906. Fig. 2

Colonies on 1/3 MEA yellow. Sporangiophores septate, erect, arising from media, simple, occasionally furcate or irregularly branched, upper portion fertile, producing sporocladia laterally, 7-10 μm diam, up to 500 μm high. Sporocladia septate, boat shaped, 32-41 × 5-7 μm, composed of 8-10 cells, terminal cell sterile, small, recurve, and rounded apically, with 1-2-celled stalk (20-56 × 5-6 μm/cell), distantly arranged on the fertile portion, one or none per cell segment of sporangiophore, nearly parallel to the axes of sporangiophores. Additional sporocladium or short sterile branch often produced from stalk. Pseudophialides plurally arising from a sporocladium, cylindrical, flask-shaped, 4-7 × 1.5-2 μm, acrogenously producing a sporangiolum. Sporangiola monosporic, elliptical, fusiform, 11-14 × 2-2.5 μm (length / wide = 5.56), rounded distally and truncate below; distal sporangial wall distinct from the apex of the sporangiospore inside. Sporangiospores fusiform, slightly tapering towards the apex, 10.5-13.5 × 2-2.5 μm, circumambient within a sporangiolum. Zygospores not observed.

Specimens examined: *ALS0501* = BCRC 34299, from soil, Chiayi County, Alishan Township, Alishan, June 2007, isol. S.C. Chuang.

Note: This species resembles *C. aciculifera* Linder

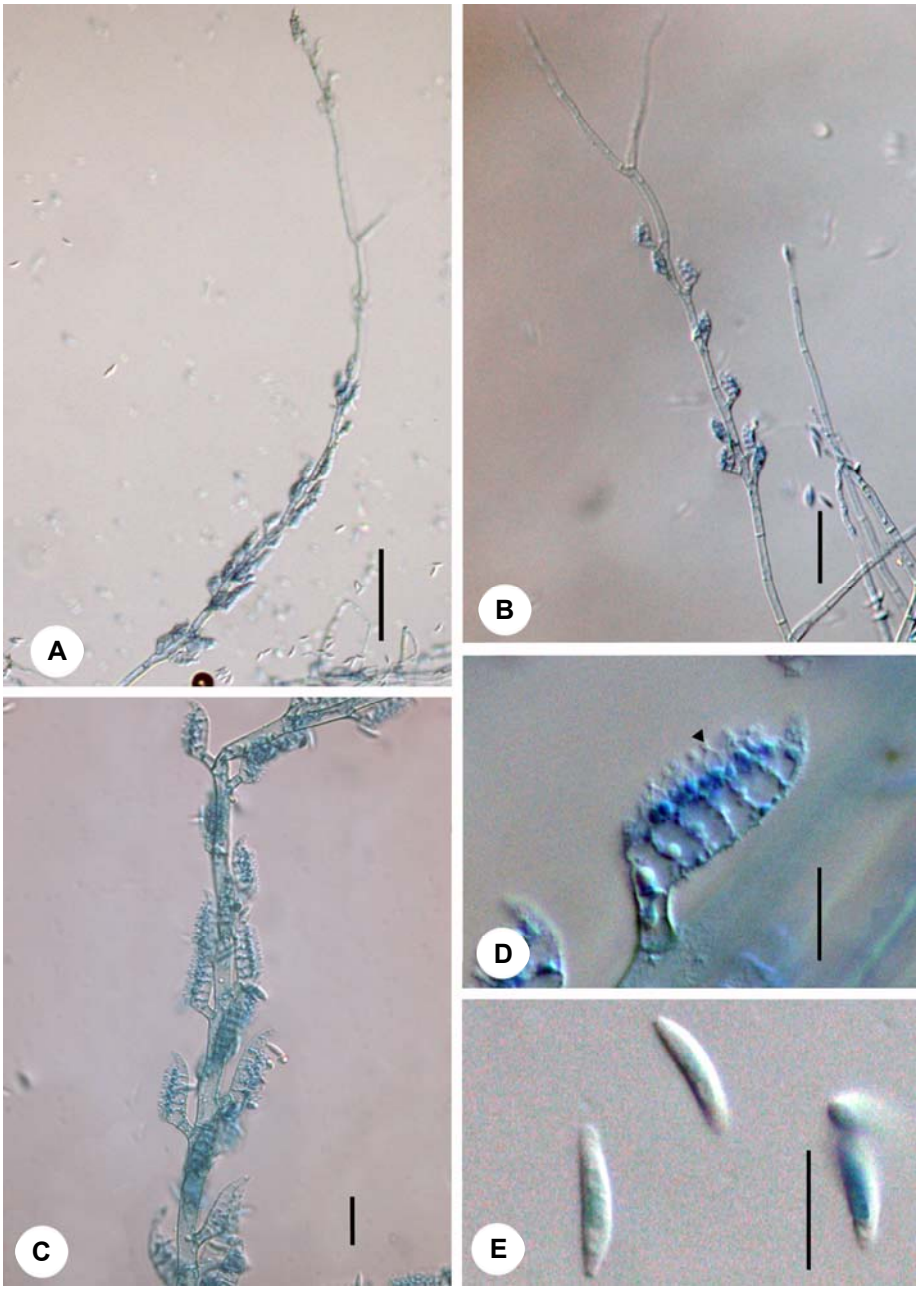

Fig. 1. *Coemansia asiatica* (*TDDCOW*). A-E: LM. A: Upper fertile portion of a sporangiophore; Bar = 50 µm. B: Fertile portion of sporangiophore with nine sporocladia; Bar = 20 µm. C: Sporangiophore with sprocladia; Bar = 10 µm. D: A sporocladium with plural flask-shaped pseudophialides (arrow head); Bar = 10 µm. E: Mature spores; Bar = 10 µm.

Table 1. GenBank accession number of three regions ribosomal DNA sequence of *Coemansia* spp.

Species	Strain number	Location	GenBank accession number		
			LSU	SSU	ITS region
Coemansia aciculifera	KYK00188	Japan	AB287993	AB287979	-
Coemansia asiatica	BTCC-F31= ID05-F0205	Indonesia	-	AB295424	AB295425
Coemansia asiatica	NBRC 102546 = Kimi	Japan	AB287996	AB287982	-
Coemansia asiatica	BCRC 34628 = *TDDCOW*	Taiwan	JN009789	JN009790	JN009791
Coemansia pectinata	BCRC 34299 = *ALS0501*	Taiwan	JN009792	JN009793	JN009794
Coemansia pectinata	IMI 142377 = RSA 2337	UK	JN009795	JN009796	JN009797

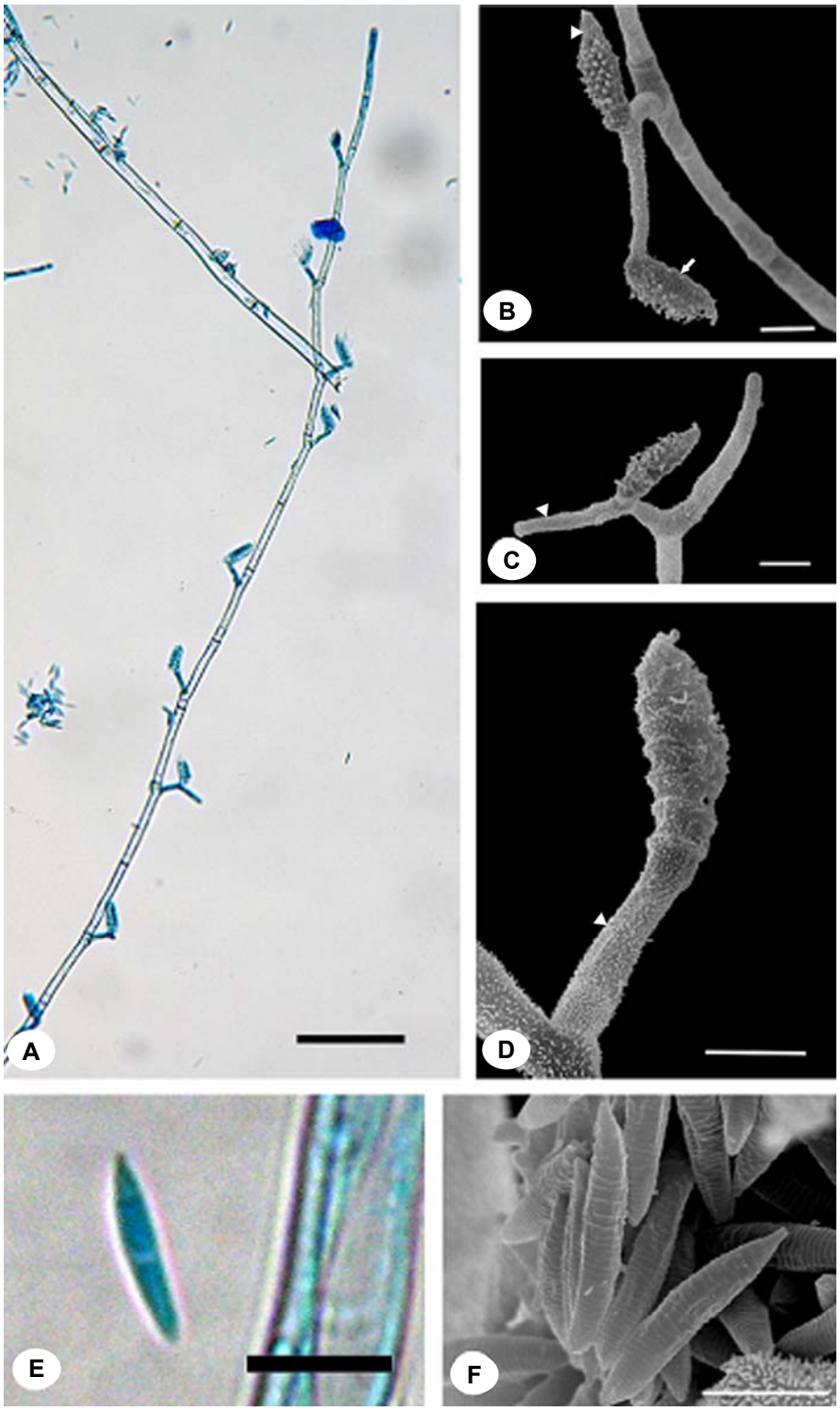

Fig. 2. ***Coemansia pectinata*** **(*ALS0501*). A, E: LM; B-D, F: SEM. A: Fertile portion of a sporangiophore showing loosely arranged sporocladia; Bar = 50 µm. B: An additional sporocladium (arrow) developing from the stalk of original sporocladium (arrow head); Bar = 10 µm. C: A proliferating branch (arrow head) arising from the stalk of original sporocladium; Bar = 10 µm. D: Back view of a sporocladium with stalk (arrow head); Bar = 10µm. E: A sporangiolum stained with cotton blue; Bar = 10 µm. F: Surface view of mature spores; Bar = 5 µm.**

and *C. thaxteri* Linder in the pattern of sporocladia arrangement on sporangiophore which is spaced distantly, and usually producing additional sporocladia or short sterile branch from the sporocladial stalk. Among them, *C. pectinata* could be distinguished from *C. thaxteri* by smaller spores (11-14 µm and 21-23.5µm, respectively), but it is more difficult to separate *C. pectinata* from *C. aciculifera* by spore size (11-14 µm and 12-16(-18) µm respectively). Nevertheless, we identified *ALS0501* as *C. pectinata* by comparing their ribosomal DNA data (Table 1). There were 12 difference (12 substitutions) in the partial sequences (632 bp) of domains D1 and D2 of the LSU rDNA between *ALS0501* and KYK00188 (*C. aciculifera*), but the former strain showed only one nucleotide difference from IMI142377 (*C. pectinata*). Moreover, *ALS0501* and IMI142377 showed three differences in the partial sequences (1681 bp) of SSU rDNA and 32 differences (30 substitutions and two idels) in ITS rDNA (1049 bp) (Table 1).

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臺灣管狀孢子囊接合菌之研究（VIII）：兩種下梳黴屬新紀錄種

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摘要：本文描述兩種臺灣產下梳黴屬接合菌 *Coemansia asiatica*（亞洲下梳黴）及 *C. pectinata*（梳狀下梳黴），分別由糞物及土壤分離，均為臺灣的新紀錄種。鑑定係基於其形態特徵及核糖體 DNA 序列分析。文中提供描述，圖示並討論其與相近種之異同。

關鍵詞：下梳黴屬、梳黴目、梳黴亞門、核糖體 DNA、臺灣、接合菌綱。