

THERMOPHILIC AND THERMOTOLERANT FUNGI IN TAIWAN (III)⁽¹⁾

GUEY-YUH CHEN⁽²⁾ and ZUEI-CHING CHEN⁽³⁾

(Manuscript received 25 January, 1991; revised accepted 25 May, 1991)

Abstract: *Emericella nidulans* (Eidam) Vuill. and *Talaromyces emersonii* Stolk are reported from Taiwan. The former is a thermotolerant fungus and the latter is a thermophilic fungus. Both were isolated by heat incubation method at 40°C from soil and wood chips.

During the course of investigation on thermophilic and thermotolerant fungi of Taiwan, seven species of thermophilic and three species of thermotolerant fungi were recognized and reported (Chen & Chen, 1988 and 1990). Additional two species, i.e., *Emericella nidulans* (Eidam) Vuill. and *Talaromyces emersonii* Stolk are treated as a thermophilic and a thermotolerant fungus, respectively in this paper. Both species have been recorded from Taiwan (Lee, 1981; Tzean, *et al.*, 1990), but lack the informations concerning their thermophilic nature. Two high temperature resistant strains were isolated from the field soils or wood chips of Taiwan, and their taxonomy, cultural morphology and response to temperature spectrum are reported here to supplements the previous descriptions (Lee, 1981; Tzean, *et al.*, 1990). The research methods followed the previous techniques (Chen & Chen, 1988).

Emericella nidulans (Eidam) Vuill., C.R. Acad. Sci. Paris 184: 137 (1927).

Anamorph: *Aspergillus nidulans* (Eidam) Wint. in Rab. Krypt.-Fl, 1(2): 62 (1884). Raper & Fennell, The genus *Aspergillus*, p. 495 (1965).

Sterigmatocystis nidulans Eidam in Cohn, Beitr. Biol. Pflanz. 3: 392-411 (1883).

Fig. 1 & Plate 1 & 2.

Colonies on PDA, attaining 83 mm in 7 days at 35°C, radiately growing, thick with abundant granules, which scattering on the surface. The color from Straw Yellow (Colour names are based on Ridgway, 1912) to Amber Yellow, reverse Ochraceous-orange. Cleistothecia globose to subglobose, 120-250 µm (-300 µm), single or in groups; the color from Pale Green, Pale Greenish Yellow, Light Greenish Yellow, Cosse Green, Lettuce Green, then turning Spinach Green; surface covered with sterile, branched hyphae and hülle cells. Hülle cells hyaline in central area, pale green or pale purple on margin, produced from the peridium hyphae, 12.4-20 µm, with 1.1-1.8 µm thick walls; Ascus globose to subglobose, with 4-8 ascospores, 6.3-8×7.3-10.6 µm. Ascospores lenticular, having two equatorial, slightly raised,

(1) This research was supported in part by National Science Council grant NSC 77-0211-B-002-01 and NSC 77-0606-B-002-167.

(2) 陳桂玉, Associate Professor, Chinese Culture University, Taipei, Taiwan 10433, Republic of China.

(3) 陳瑞青, Professor, Department of Botany, National Taiwan University, Taipei, Taiwan 10764, Republic of China.

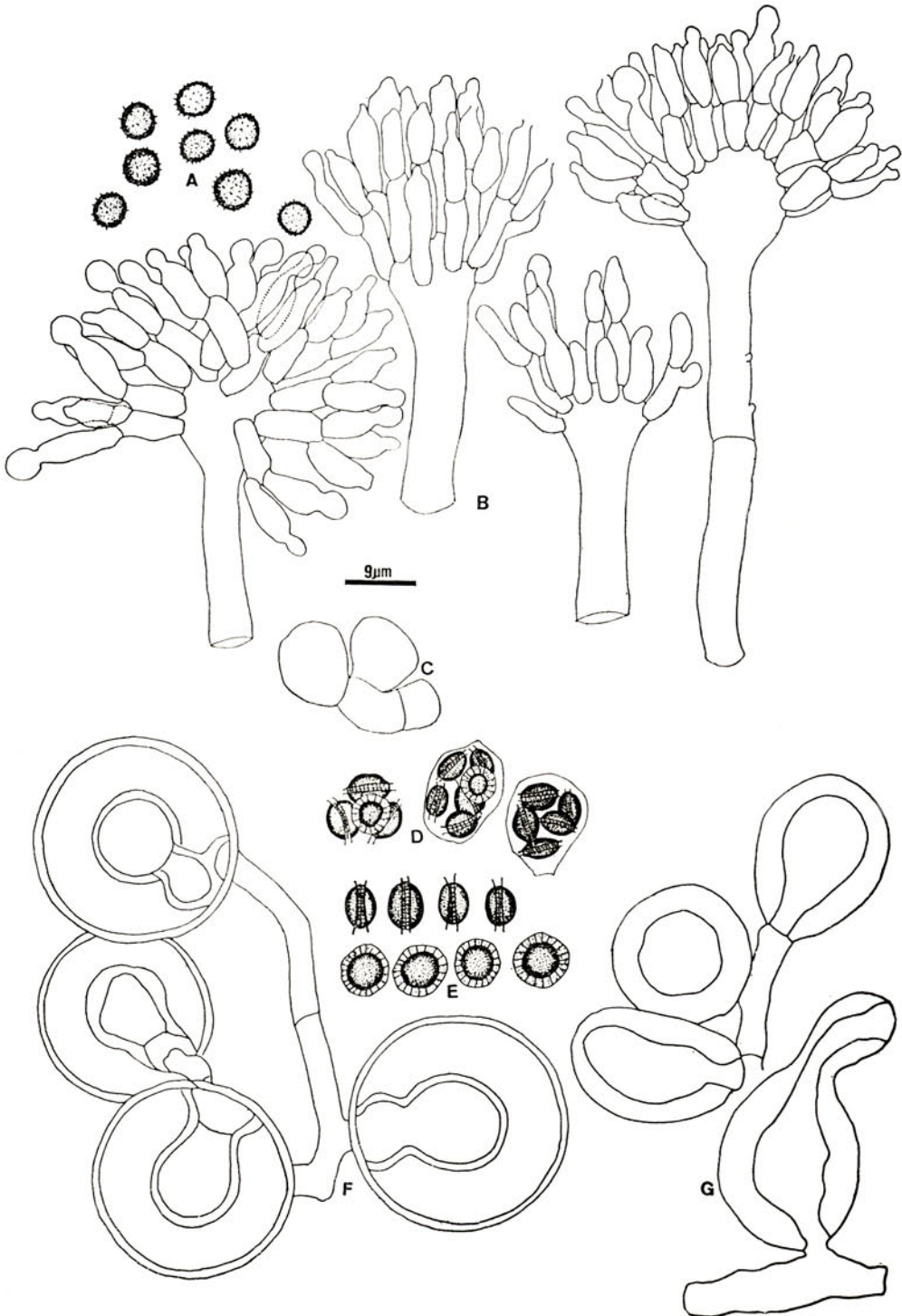


Fig. 1. *Emericella nidulans* (Eidam) Vuill. A: Conidia; B: Conidiophores; C: Initial asci; D: Asci with ascospores; E: Ascospores; F: Spherical hülle cells at 25-30°C; G: Irregular hülle cells at 16-20°C.

furrowing crests with smooth convex surface, $2.8-3.4 \times 4.6-5.2 \mu\text{m}$, crest about $0.6 \mu\text{m}$ in width, the interval of two crests $0.5-0.9 \mu\text{m}$.

Conidiogenesis: Conidial head dark green with short column, $29-41.5 \times 50 \mu\text{m}$; Conidiophores septate, pale brown, smooth, produced from aerial or substrate mycelia, $4.2-5.6 \times 53-148 \mu\text{m}$; vesicle pale brown, hemispherical to spherical, $9-11.8 \mu\text{m}$; phialide loosely arranged, mainly biserial, few uniserial, metulae $2.8-4.4 \times 5.6-8.4 \mu\text{m}$, phialides $2.6-3.4 \times 6.7-10.6 \mu\text{m}$; Conidia pale green, globose to sub-globose, $3.0-4.5 \mu\text{m}$, wall echinulate under light microscope. lobate-reticulate under SEM. (Plate 1. E & F).

MEA: Attaining 83 mm in 7 days at 35°C . Colonies thin, sparse with asexual structure and granules scattering on the surface; reverse Naples Yellow, Yellowish Citrine, Serpentine Green, Mustard Yellow, then White in color.

CZA: Attaining 79 mm in 7 days at 35°C . Colonies forming a layer, furrowing; the color from Cinnamon-Buff, Lemon Chrome, and Lettuce Green; reverse Bay, Sanfords Brown, Pale Maize Yellow in margin. Ascocarps extremely few or none, having asexual structure.

Specimens examined: Taiwan. Pingtung Hsien: Changchih Hsiang, Low land, Field soil, VIII. 1986. Chen G-Y 8608-3 (TAI).

Temperature tests: As shown in Table 1. Mycelia growth on PDA and MEA were faster than on CZA and an optimum growth temperature was observed at 35°C on all media tested. The conidial structures were observed from 16°C to 50°C in all media tested except PDA and MEA at 50°C . In contrast, formation of ascomata was rather restricted and no ascocarp was produced in CZA at all range of temperature tested. However, on both PDA and MEA, formation of fertile ascomata were observed from 25°C to 40°C with best results on 30°C – 35°C . Below 20°C , no fertile ascocarp was obtained but only sterile ascocarps with irregular hülle cells were observed. At 50°C , both sexual and asexual reproduction were greatly suppressed and only mycelial growth was noticed.

Note: *E. nidulans* has been described from Taiwan by Tzean, *et al.* (1990) based on growth morphology at room temperature (25°C). Our isolate though identical to *E. nidulans*, has an optimum growth temperature at 35°C and can grow

Table 1. Growth and morphology of *E. nidulans* at different temperatures

Temp. ($^\circ\text{C}$)		16	20	25	30	35	40	45
Medium		Diameter of colony in mm.						
Mycelial growth	PDA	13	33	38	75	83	37	16
	MEA	14	32	42	80	83	38	12
	CZA	12	23	34	70	79	34	11
Ascomata	PDA	—	—	††	‡‡	‡‡	+	—
	MEA	—	—	+	††	††	+	—
	CZA	—	—	—	—	—	—	—
Conidial structure	PDA	+	+	††	‡‡	‡‡	††	—
	MEA	+	+	+	††	††	+	—
	CZA	+	+	††	‡‡	‡‡	††	+

Diameter of petri dish: 85 mm.

Ascomata & Conidial structure: (—) none produced, (+) moderate, (††) abundant, (‡‡) very abundant.

at 16°C and 50°C. Evidently it belongs to a thermotolerant fungus. The description of this isolated was based on growth morphology at 35°C, which may slightly differ to the description based on room temperature. The surface structure of conidia under the light microscope was echinulate but under SEM, it showed lobate-reticulate wall which is identical to the structure reported by Kozakiewicz (1989).

Talaromyces emersonii Stolk, Antonie van Leeuwenhoek, 31: 262 (1965); Stolk & Samson, The genus *Talaromyces*, p. 48 (1972).

Anamorph: *Penicillium emersonii* Stolk, Antonie van Leeuwenhoek, 31: 262 (1965).
Fig. 2 & Plate 3.

Colonies on PDA, growing very well, reaching whole plate in 7 days at 40°C, forming abundant ascocarps and mixed with conidial structures; the color first

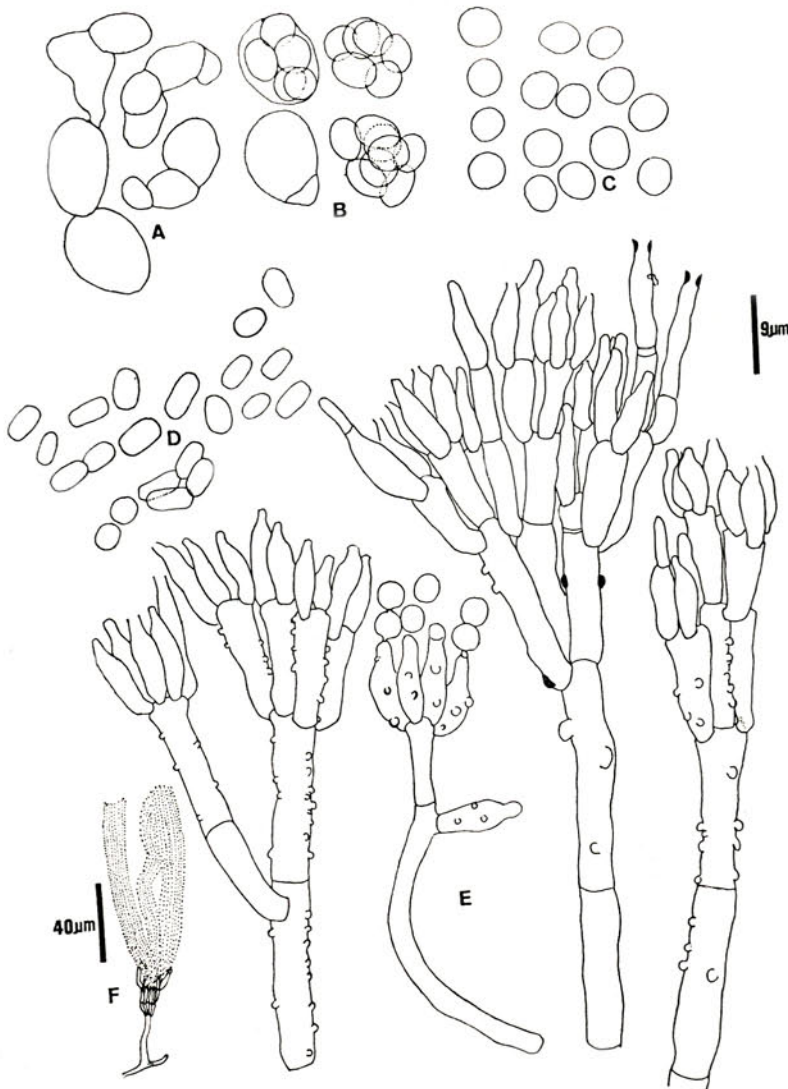


Fig. 2. *Talaromyces emersonii* Stolk. A: Initial asci; B: Asci with ascospores; C: Ascospores; D: Conidia; E: Conidiophores; F: Habit Sketches.

White and Pale Yellow, then turning Orange; reverse Orange, Empire Yellow and Pirard Yellow.

Ascomata forming a layer, white to pale yellow and orange by age; hyphae loosely covering ascomata without peridium, easily crushed, 40–140 (–200) μm . Ascus in chains with 4–8 ascospores, subglobose to elliptical, 6.3–8.0 \times 7.0–9.1 μm ; Ascospores yellowish brown, smooth, globose, subglobose or ovoidal, 2.3–3.7 \times 3.4–4.0 μm .

Conidiogenesis: Conidiophore produced from aerial or prostrate mycelium, septate, wall verrucose to smooth, bearing terminal penicilli, 2.8–44.5 \times 15.5–60 μm . Penicilli monoverticillate, biverticillate-asymmetrical and irregularly branching. Phialides 3–5, yellowish brown with smooth or slightly roughened walls, 2.3–2.8 \times 8–10.8 μm ; Metulae 2–5, with roughened walls, 2.9–6.3 \times 10.3–15.4 μm ; Rami 2–3, pale gray, wall roughened, 3.1–3.4 \times 17.7–21.9 μm . Conidia pale yellow, smooth globose to subglobose, (2.6–2.9 μm in diam), elliptical (2.3–2.9 \times 2.8–4.4 μm), or cylindrical (1.8–2.9 \times 3.4–4.7 μm).

Specimens examined: Taiwan. Nantou Hsien: Puli Chen, Wood chips, X. 1984. Chen G-Y 8410-1. (TAI). Taitung Hsien: Taitung, Field soils. IX. 1987. Chen G-Y 8709-6. (TAI).

Temperature tests: The present isolates of *T. emersonii* could not grow at all media tested below 30°C. Growth rate of mycelia on CYA was slower than those of PDA and MEA at high temperatures range between 40°C–50°C, which were also the optimum growth temperatures of present isolates. Ascomata were abundantly produced on PDA and MEA at 35°C and 40°C and none on CYA medium. In contrast, conidia were abundantly produced at the same temperature range on CYA and less in both PDA and MEA. At 50°C, although no ascomata was formed in all media tested, the optimum growth of mycelia and abundance formation of conidia were observed.

Note: The isolates described here were identified based on an original description of Stolk (1965) and are the typical thermophilic fungi. No growth below 30°C and the phenomena of development of other temperatures were identical to descriptions of Awao & Otsuka (1973). The apices of metulae or phialides were swollen at 50°C. In most cases, ascomata first turned to yellow, then to orange,

Table 2. Growth and morphology of *T. emersonii* at different temperatures

Temp. (°C)		20	25	30	35	40	50
Medium		Diameter of colony in mm.					
Mycelial growth	PDA	*	*	*	39	85	85
	MEA	*	*	*	41	85	85
	CYA	*	*	*	32	39	30
Ascomata	PDA	*	*	*	+++	+++	—
	MEA	*	*	*	+++	+++	—
	CYA	*	*	*	—	—	—
Conidial structure	PDA	*	*	*	+	+	++
	MEA	*	*	*	+	+	++
	CYA	*	*	*	+++	+++	++

Diameter of petri dish: 85 mm. (*): no growth

Ascomata and conidial structure: (—) none produced, (+) moderate, (++) abundance, (+++) very abundance.

except a few plates without color change and keep yellow color throughout. This character was identical with the strain of Evens (1971), which may be a mutant form. Lee (1981) isolated this species from rice straw etc. from Taiwan and studied the production of cellulase of this fungus without any morphological description. The present description is intended to supplement this need.

LITERATURE CITED

- AWAO, T. and S.I. OTSUKA, 1973. Note on thermophilic fungi in Japan (2). Trans. mycol. Soc. Japan **14**: 221-236.
- CHEN, G. Y. and Z. C. CHEN, 1988. Thermophilic and thermotolerant fungi in Taiwan (I). Trans. mycol. Soc. ROC. **3**(1): 1-72.
- CHEN, G. Y. and Z. C. CHEN, 1990. Thermophilic and thermotolerant fungi in Taiwan (II). Taiwan **35**: 191-197.
- EVENS, H. C., 1971. Thermophilous fungi of coal spoil tips I. Taxonomy. Trans. Br. mycol. Soc. **57**(2): 241-254.
- KOZAKIEWICZ, Z., 1989. *Aspergillus* species on stored products. Mycological papers (6): 188 p.
- RIDGWAY, R., 1912. Color Standards and color nomenclature. publ. by the author. Washington, D. C. 43 p.
- STOLK, A. C., 1965. Thermophilic species of *Talaromyces* Benjamin and *Thermoascus* Miehe. Antonie van Leeuwenhoek **31**: 262-276.
- LEE, C. Y., 1981. Studies on cellulases produced by thermophilic fungi. National Taiwan University, Graduate Institute of Agricultural Chemistry. Master Thesis.
- TZEAN, S. S., J. L. CHEN, G. Y. LIOU, C. C. CHEN and W. H. HSU, 1990. *Aspergillus* and related teleomorphs from Taiwan. Mycological Monograph No. 1, Food Industry Research and Development Inst., Hsinchu, Taiwan. p. 83-85.

臺灣嗜熱性和耐熱性真菌之調查 (III)

陳桂玉 陳瑞青

摘 要

此篇報告臺灣產兩種子囊菌。 *Emericella nidulans* (Eidam) Vuill. 及 *Talaromyces emersonii* Stolk。前者為耐熱性真菌，自臺灣之屏東縣長治鄉田土所分離出來。後者為嗜熱性真菌，自埔里菇場之堆積木屑及臺東市田土分離的。

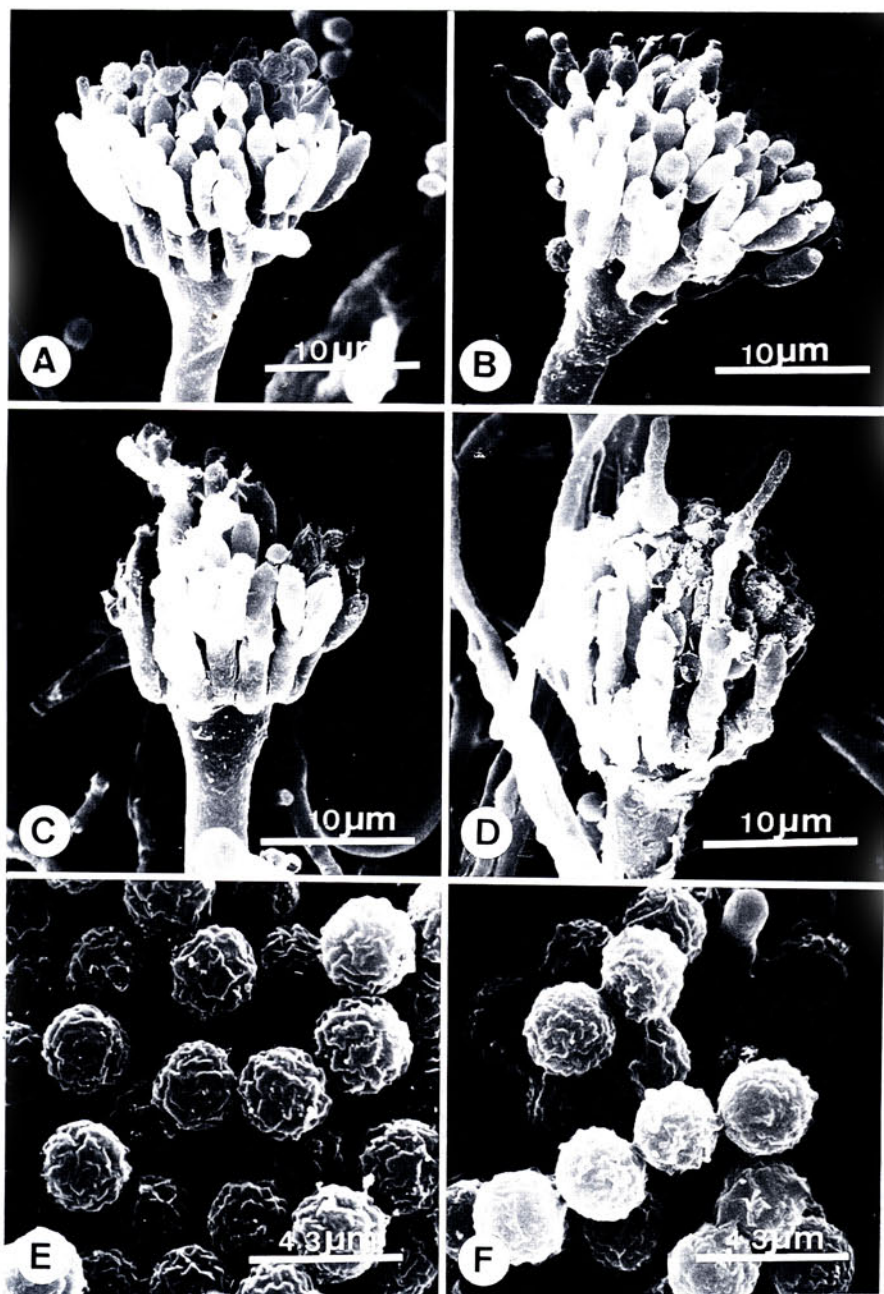


Plate 1. *Emericella nidulans* (Eidam) Vuill.

A-D: Conidial head.

A: Biseriate sterigmata at 25°C.

B: Biseriate sterigmata at 35°C.

C & D: Irregular growth of phialide at 40°C.

E-F: Conidia.

E: At 30°C.

F: At 40°C.

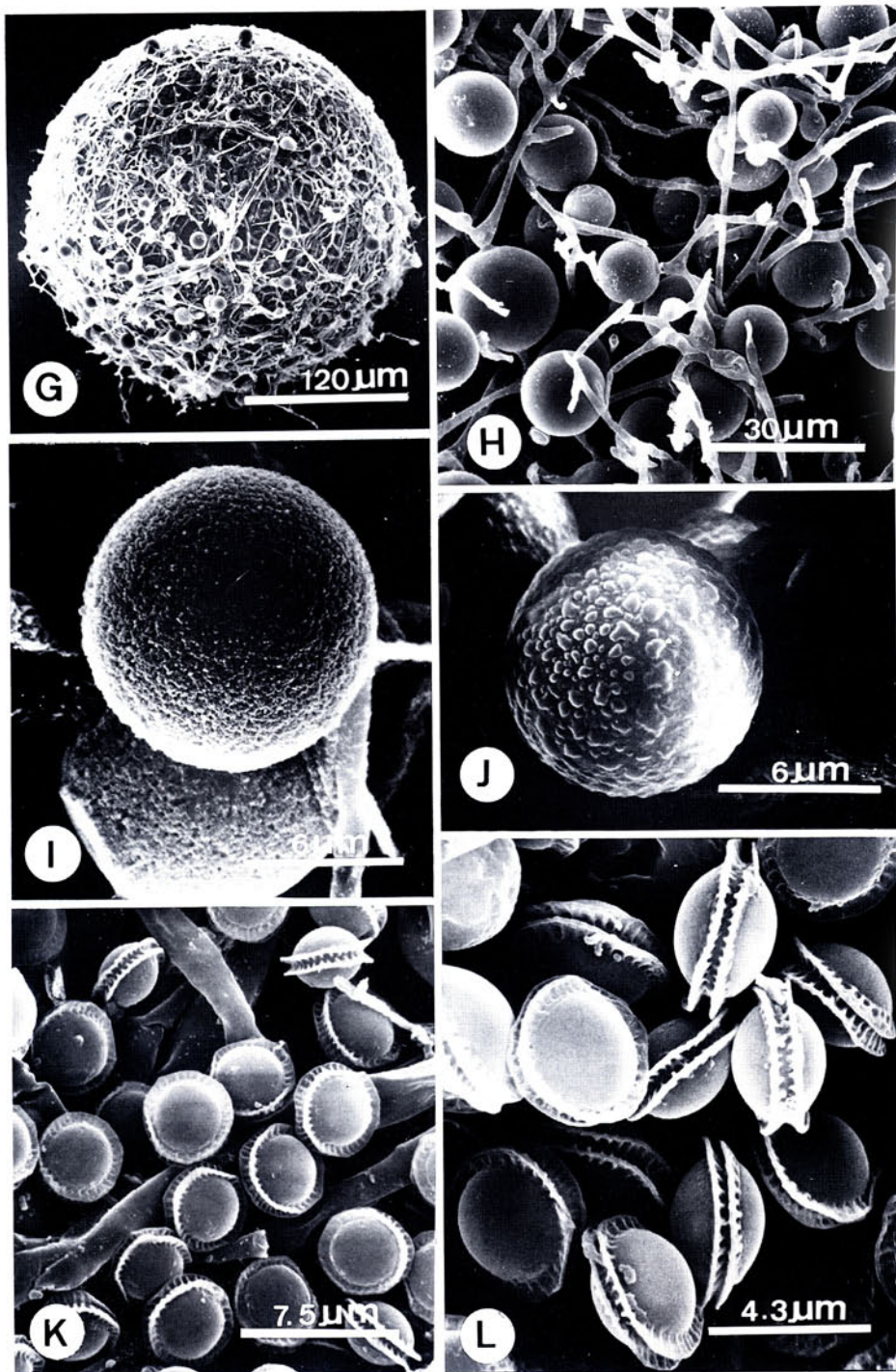


Plate 2. *Emericella nidulans* (Eidam) Vuill.

G-J: Hülle cells.

K-L: Ascospores.

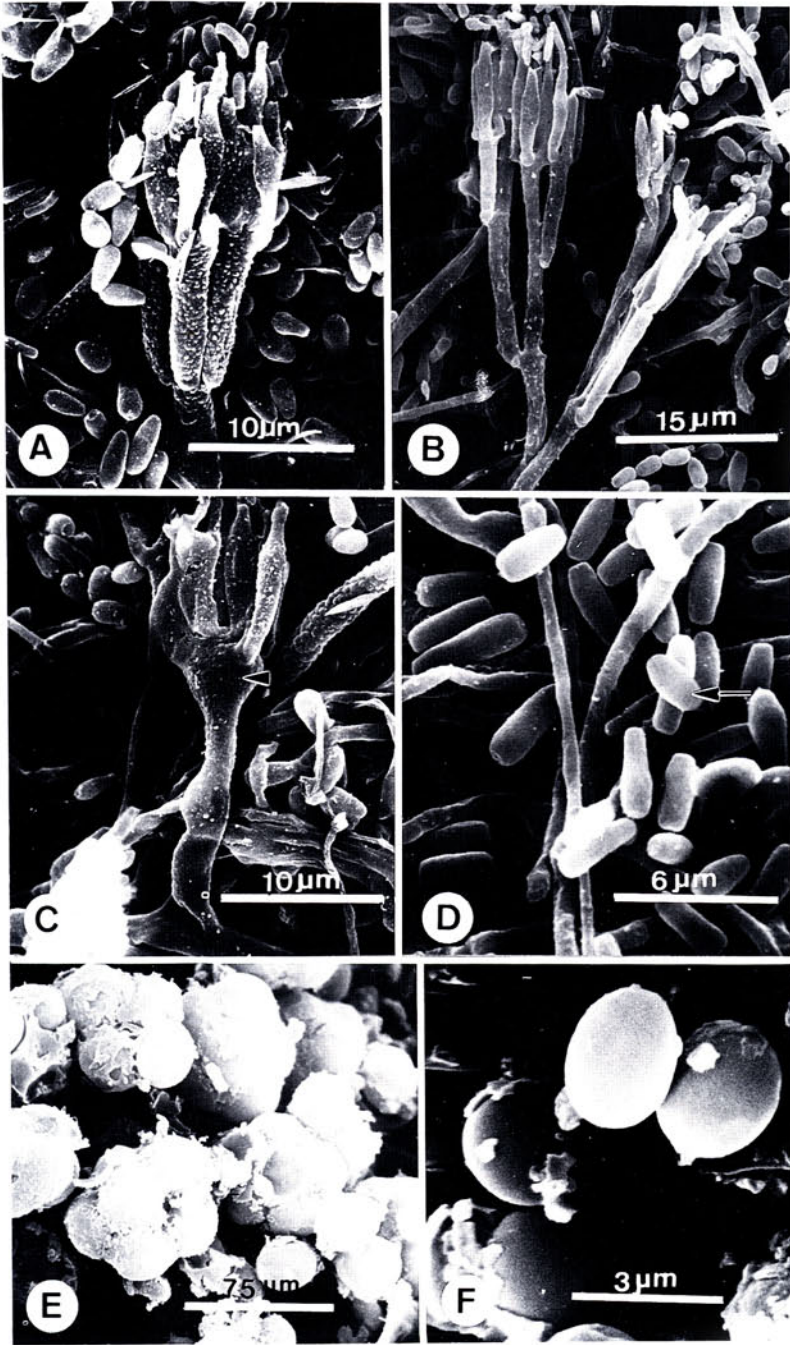


Plate 3. *Talaromyces emersonii* Stolk.

A-C: Conidial head.

A: The rough wall of stipe and sterigma.

C: The swelling apex of a stipe (arrow).

E: Non-separate ascospores.

F: Ascospores.