

Two interesting cleistothecial Ascomycetes from soils

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Accepted for publication 28 February 1995

A new variety of *Thermoascus*, *Thermoascus crustaceus* var. *verrucosus*, is described and illustrated. It is characterized by producing prominently verrucose ascospores. The other characters are almost identical with those of the type variety. *Leucothecium coprophilum*, a rare ascomycete that has only previously been reported from Spain, is recorded from soils in Taiwan and Korea.

Key Words—China; Korea; *Leucothecium coprophilum*; soil fungi; Taiwan; *Thermoascus crustaceus* var. *verrucosus*.

During our continuous survey of soil-borne fungi as producers of fungal metabolites useful to the pharmaceutical industry, two interesting cleistothecial Ascomycetes have been isolated from soils in China, Taiwan and Korea. The first fungus is a new variety of *Thermoascus crustaceus* (Apinis et Chesters) Stolk in the Eurotiales, of which ascospores are verrucose. The second fungus is determined as *Leucothecium coprophilum* in the Onygenales, known only from Spain (Valldosera et al., 1991). Color nomenclatures are from the Kornerup and Wanscher (1978) color handbook and the Rayner (1970) color chart, and are referred to with the letters M and R, respectively.

***Thermoascus crustaceus* (Apinis et Chesters) Stolk, var. *verrucosus* Yaguchi, Someya et Udagawa, var. nov.**
Fig. 1(A–F)

A typo differt ascosporis verrucosis.

Holotypus PF 1160, colonia exsiccata in cultura ex solo, Guanghou, in Sina, 4. xi. 1993, a T. Yaguchi isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: from Latin, *verrucosus*=verrucose, referring to the character of ascospores.

Anamorph: *Paecilomyces crustaceus* Apinis et Chesters pro parte.

Colonies on Czapek agar spreading broadly, attaining a diam of 80 mm in 7 days at 37°C, more or less floccose, plane, thin, vegetative mycelium submerged; ascomata moderately produced on the substratum, overgrown by sparse aerial hyphae, Greyish Red (M. 7B4) or Rosy Buff (R) in color; margins irregular, thin; conidiogenesis limited in marginal areas, not sufficiently produced to influence the colony appearance; reverse Reddish Brown (M. 8E6) or Rust (R).

Colonies on Czapek-yeast extract agar (CYA) spreading broadly, attaining a diam of 80 mm in 7 days at 37°C, consisting of a rather compact basal felt, wrinkled

throughout the colony, becoming granular in appearance due to the production of numerous ascomata, Orange (M. 6A6) or Saffron (R) in color, covered with flocculent aerial hyphae; margins more or less irregular, thin; conidiogenesis sparse; exudate profuse, clear; reverse Reddish Brown (M. 8E7) or Rust (R).

Colonies on malt extract agar (MEA) spreading broadly at 37°C, in growth-rate as on CYA, thin, vegetative mycelium submerged; ascomata radially produced on the substratum, intermixed with sparse conidiophores and conidia, Pastel Red (M. 7A4) or Peach (R) in color; margins entire, thin; reverse Reddish Brown (M. 9D7) or Rust (R).

Colonies on oatmeal agar (OA) spreading broadly at 37°C, in growth-rate as on CYA, plane, consisting of a thin basal felt, with surface granular in appearance as on CYA, producing abundant ascomata, Brownish Orange (M. 5C4) or Honey (R); conidiogenesis inconspicuous, not affecting the colony appearance; exudate abundant, clear; reverse Greyish Yellow (M. 4B4) or Pale Luteous (R).

Ascomata superficial, often confluent in clusters, non-ostiolate, orange, hemispheroidal, 350–500 µm or more in diam; peridium membranaceous, of textura angularis to textura globosa; outer layer consisting of yellow, more or less thick-walled, angular cells measuring 4–8 × 2–6 µm; inner layer of hyaline, angular or rounded, 10–20 µm diam cells. Ascumata initials of a coiled hypha. Asci singly borne, usually 8-spored, globose to pyriform, 12–16 × 11–14 µm, often short-stipitate, evanescent. Ascospores hyaline to pale reddish orange, ellipsoidal, 6–8 × 5–6 µm, verrucose, thick-walled.

Conidiophores arising from the basal mycelium or as lateral branches of aerial hyphae; stipes up to 350 µm long and 6–10 µm in diam, hyaline to brownish, septate, with walls smooth to finely roughened. Penicilli comprising very irregular verticils of terminal and subterminal metulae or rami, sometimes with concurrent phialides; met-

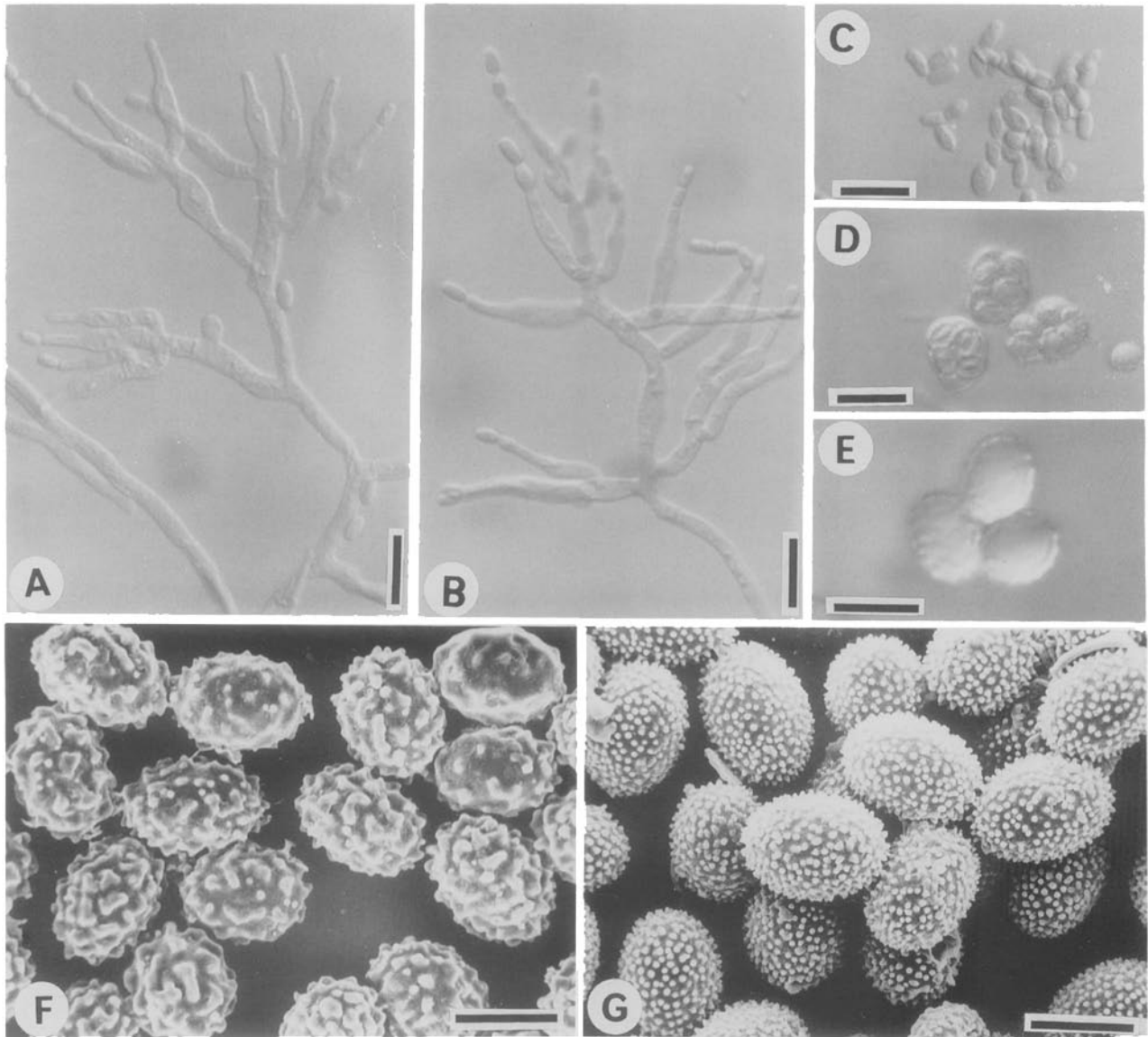


Fig. 1. *Thermoascus crustaceus* var. *verrucosus*, PF 1160. A, B. Conidiogenous cells. C. Conidia. D. Asci. E. Ascospores (LM). F. Ascospores (SEM).

Thermoascus crustaceus var. *crustaceus*, IFO 31853. G. Ascospores (SEM).

Scale bars for A to D = 10 μm and for E to G = 5 μm .

ulae often in irregular verticils, 10–18 \times 5–8 μm , smooth-walled; phialides in verticils of 2–6, 16–30 \times 4–6 μm , cylindrical, sometimes swollen up to 14 μm in diam, smooth-walled, variable in shape, one ellipsoidal to cylindrical, truncated at both ends, 4–10 \times 2–4 μm , other ovoid to pyriform or subglobose, 5.5–8 \times 5–6 μm , both borne in long disordered chains. Chlamydospores not seen.

Specimen examined: PF 1160 (holotype), in dried culture isolated from soil, Guangzhou, China, 4 November 1993, coll. T. Yaguchi. The holotype has been deposited with the Natural History Museum and Institute, Chiba, Japan (CBM).

Thermoascus crustaceus var. *verrucosus* is readily distinguished from the variety *crustaceus* (Stolk, 1965;

Udagawa et al., 1973; Awao and Otsuka, 1974) and *T. aegyptiacus* Ueda et Udagawa (Ueda and Udagawa, 1983) by ascospore ornamentation (see Fig. 1-F, compared with G), but other characters show only minor discrepancies from the type variety.

Leucothecium coprophilum Valdsoera et Guarro, Mycol. Res. 95: 243. 1991. Fig. 2

Colonies on CYA growing slowly, attaining a diam of 30 mm in 1 month at 25 $^{\circ}\text{C}$, plane, thin, consisting of submerged vegetative mycelium, with surface very sparse white aerial hyphae and conidia, at the center White to somewhat Yellowish White (M. 3A2) or Primrose (R); ascromata limited in submarginal areas, not affecting the colony appearance; exudate small, clear; odor somewhat

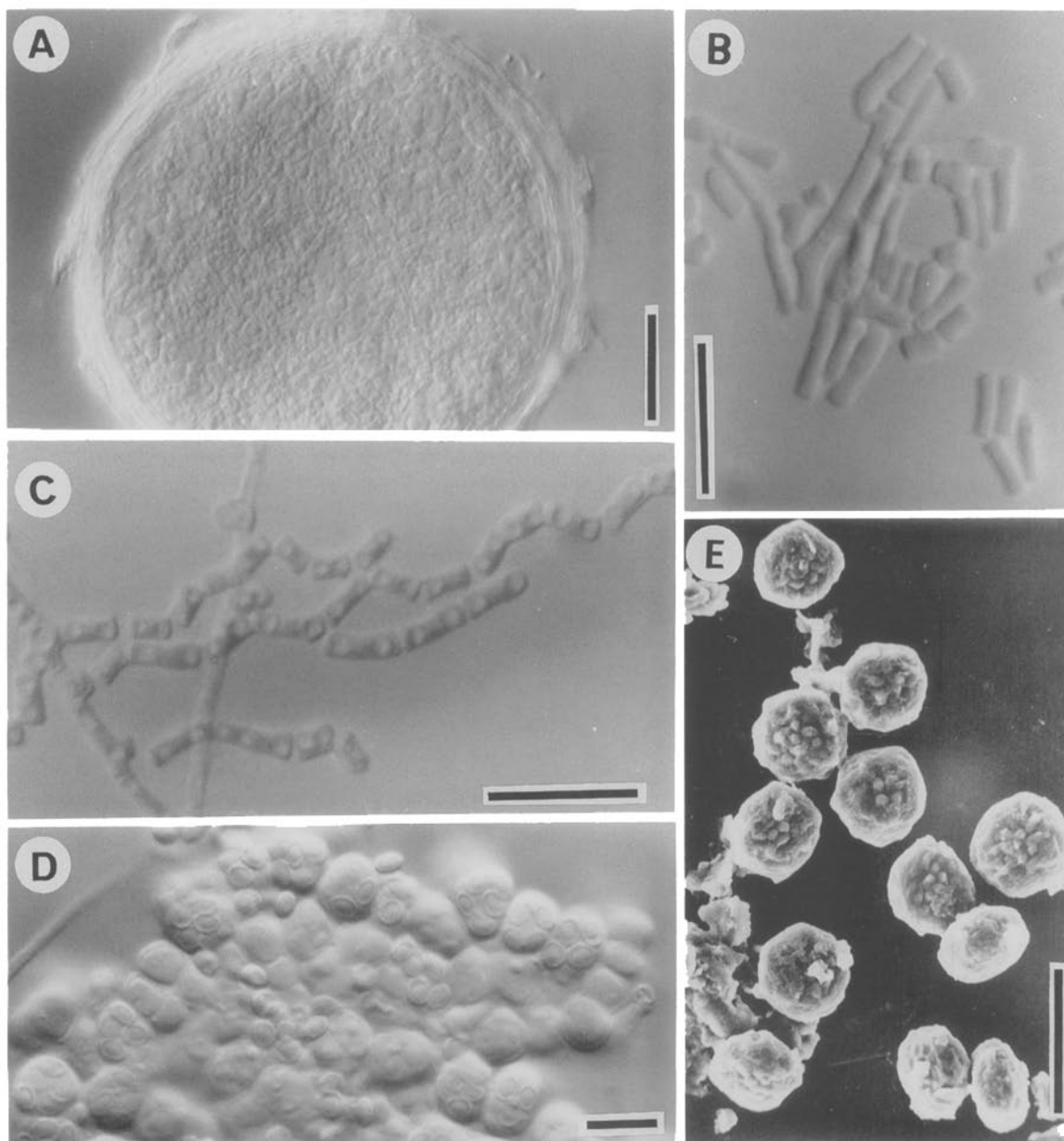


Fig. 2. *Leucothecium coprophilum*, PF 1161. A. Ascoma. B, C. Arthroconidia. D. Asci. E. Ascospores (SEM). Scale bars for A = 100 μm , for B, C = 10 μm and for D, E = 5 μm .

unpleasant; reverse uncolored.

Colonies on MEA growing slowly at 25°C, in growth-rate as on CYA, plane, thin, mostly wettish, with very sparse aerial growth, Yellowish White (M. 2A2) or Primrose (R); ascomata not developed within 1 month; margins very irregular; reverse uncolored.

Colonies on OA growing slowly, attaining a diam of 25 mm in 1 month at 25°C, plane, thin, vegetative mycelium almost submerged, with surface somewhat wettish; yellowish ascomata irregularly scattered on the agar surface, almost uncovered with aerial growth, somewhat aggregated at the center, Pastel Yellow (M. 3A4) or Pure Yellow (R); margins thin, irregular; conidiogenesis limited on somewhat tomentose hyphae, not influencing the colony appearance; reverse Pale Yellow (M. 3A3) or

Straw (R).

Colonies on cornmeal agar growing slowly at 25°C, in growth-rate as on CYA, plane, thin, vegetative mycelium submerged, somewhat floccose, producing rather abundant ascomata on the substratum or into the agar, white, not affecting the colony appearance; conidiogenesis not conspicuous, white; reverse uncolored.

Ascomata superficial to semi-immersed, scattered or aggregated in small clusters, pale yellow, subglobose, depressed above, variable in size, 375–575 μm high, 450–750 μm in diam, covered with agglutinated cells (chlamyospore-like cells) measuring 5–10 μm in diam, rough in appearance, non-ostiolate; peridium 37.5–50 μm thick, soft, of textura angularis; outer layer consisting of pale yellow, angular cells measuring 3–7.5 μm

in diam; inner layer of hyaline, flattened cells. Asci irregularly disposed, slowly developing, 8-spored, hyaline, subglobose to ovoid, $4\text{--}5.5 \times 4\text{--}4.5 \mu\text{m}$, evanescent at maturity. Ascospores hyaline, pale yellow in mass, globose to subglobose, ellipsoidal in side view, $2\text{--}2.5 \times 2\text{--}2.5 \times 1.5\text{--}2 \mu\text{m}$, finely verrucose (after SEM).

Mycelium composed of hyaline, branched, septate, smooth-walled, $2\text{--}2.5 \mu\text{m}$ diam hyphae. Arthroconidia borne by fragmentation as a lateral branch on aerial hyphae, hyaline, white in mass, smooth-walled; terminal one subglobose to pyriform, $2.5\text{--}6 \times 2\text{--}3 \mu\text{m}$, truncated at the base, rounded at the apex; internal one cylindrical or bacilliform, $2.5\text{--}9 \times 1\text{--}2 \mu\text{m}$, truncated at the both ends, straight or somewhat curved.

At 37°C , growth is nil.

Specimens examined: PF1161, a dried culture derived from an isolate of soil, Ali Shanmo, Taiwan, 9 March 1994, collected by S. Udagawa; and PF 1162, from soil, Soul, Korea, 26 July 1991, collected by T. Yaguchi.

The genus *Leucothecium* was erected on a single species, *L. emdenii* von Arx et Samson, by von Arx and Samson (1973), who considered the genus to be closely related to *Dichotomomyces* Saito ex Scott. Later von Arx (1987) placed the genus in the family Gymnoascaceae, whereas Benny and Kimbrough (1980) regarded it as a member of the Onygenaceae. This genus, although excluded from the Onygenales by Currah (1985), is considered under the Onygenales in most treatments (Eriksson and Hawksworth, 1993).

Leucothecium coprophilum, the second species, has been reported only from goat and sheep dung in Spain (Valldosera et al., 1991). It is readily identified in having yellowish lenticular ascospores (smaller than those of *L. emdenii*, which measure $2.5\text{--}3 \times 1.7\text{--}2.3 \mu\text{m}$), arthroconidia and thick-walled cells surrounding the non-

ostiolate ascomata. The Asian strains differ from the Spanish in some minor features, such as their lack of growth at 37°C and longer arthroconidia ($2.5\text{--}9 \times 1\text{--}2 \mu\text{m}$ vs. $2.5\text{--}5 \times 1.5\text{--}2 \mu\text{m}$).

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