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TALAROMYCES UNICUS SP. NOV. FROM TAIWAN

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ABSTRACT

Talaromyces unicus and its anamorph *Penicillium unicum* isolated from preheated soil are described as a new species. The teleomorph is characterized by soft, pelliculate yellow ascocarps, slightly curved ascal chains, spinose ascospores with a monoequatorial flange or X-, Y-, spiral-, or cross-shaped, irregularly disposed flanges. The anamorph is characterized by mono- or biverticillate penicilli with long necked lanceolate phialides. The distinction between *T. unicus* and some closely related species, viz. *T. leycettanus, T. luteus, T. ohiensis, T. stipitatus, T. thermophilus,* and *T. udagawae* is discussed.

Key Words: Hyphomycetes, *Penicillium,* Plectomycetes, *Talaromyces,* taxonomy

Talaromyces (type species = *T. vermiculatus* (Dang.) Benj.) was erected by Benjamin (**1955**) to accommodate 10 species. It differed from the closely related *Eupenicillium* by producing soft gymnothecia instead of hard sclerotioid ascomata (Stolk and Samson, **1983**). Nevertheless, *Talaromyces* was considered to be heterogeneous. Its circumscription was later redefined and restricted to species that produce asci in chains (Stolk and Samson, **1972**). Two species *T. avellaneus* (Thom & Turesson) Benj. and *T. striata* (Raper & Fennel) Benj., which produced an ascus singly from a crozier, were excluded and recombined into the new genus *Hamigera* (Stolk and Samson, **1971**). Twenty validly published *Talaromyces* species, except *T. derxii* Takada & Uakada, are all homothallic (Stolk and Samson, **1972**; Pitt, **1979**; Takada and Udagawa, **1988**; Samson and Mahoney, **1977**; Samson and Abdel-Fattah, **1978**; Frisvad et al., **1990**). They have *Penicillium, Geosmithia, Merimbla,* or *Paecilomyces* anamorphs (Pitt, **1979**).

An unusual *Talaromyces* was recovered from preheated soil in Taiwan. One of the most distinguishing characteristics of this isolate is the ornamented spinose ascospores with a single equatorial or irregularly disposed flanges. This isolate was distinct from any previously described species (Stolk and Samson, **1972**; Pitt, **1979**). It is therefore described as a new taxon *T. unicus* with *P. unicum* as its anamorph. The morphology and structure, particularly on ascogenesis, are discussed.

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MATERIALS AND METHODS

An unusual *Talaromyces* was isolated from a soil sample from Funlu, Chiayi County, Taiwan, June 28, 1990. This soil sample had been suspended in a 60 C aqueous solution for 20 min before being added to dichloram rose bengal chloramphenicol (DRBC) agar plates (King et al., **1979**). This *Talaromyces* had unique ornamentations on the ascospores and appeared to be distinct from known species. The fungus was inoculated onto Czapek's yeast agar (CYA), malt-extract agar (MEA), G25N (Pitt, **1979**), and oat meal agar (OMA) (40 g rolled oat, 15 g agar powder, 1 L distilled water), incubated and examined following the standard procedures set by Pitt (**1979**). The emended circumscription and the key references on *Talaromyces* by Stolk and Samson (**1972**), Pitt (**1979**), Samson and Abdel-Fattah (**1978**), Samson and Mahoney (**1977**), Stolk and Samson (**1971**), Takada and Udagawa (**1988**), and Frisvad et al. (**1990**) were used to identify this fungus. Comparisons of morphological characteristics of this fungus with similar species relied solely on published descriptions mentioned above. The morphology and structure of the fungus was studied with scanning electron microscopy using methods described previously (Tzean and Estey, **1978**). The Kornerup and Wanscher (**1978**) color standard was used.

RESULTS AND DISCUSSION

Talaromyces unicus Tzean, Chen *et* Shiu, *sp. nov.* Stat. anamorph **Penicillium unicum** Tzean, Chen *et* Shiu, *sp. nov.* FIGS. 1–28

Coloniae in MEA, 25 C, 7 diebus, 15–19.2 mm diametri, densae, floccosae, villosae vel funiculosae; margines inferni, fibrillosi; mycelia alba ad flavida alba; ascomata discreta vel raro confluentes, suspensa in aeriis hyphis vel immersis, intertextis intra mycelia matrices; conidiogenesis moderata, alba; solubile pigmentum pallidum flavum; reversum pallidum flavum, flavum vel clarum aurantiacum, atrum aurantiacum ad rubens aurantiacum. Gymnothecia lenia, abunda, globosa, subglobosa vel ellipsoidea, pallida flava vel flava, 280–

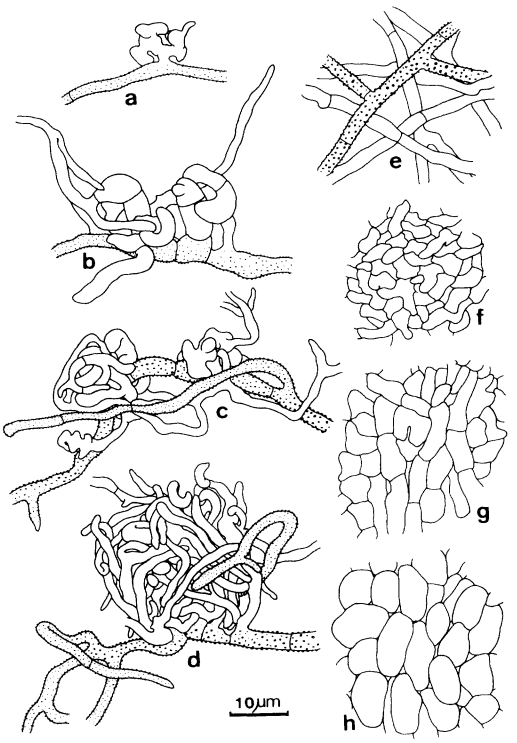


FIG. 1. *Talaromyces unicus*. a. Initial. b–d. Initials swelling, septating, coiling, branching, fusing and finally developing into ascogenous hyphae. e–h. Gymnothecial wall pelliculate, separating into three layers, outermost textura epidermoidea (f) surrounded by loose, encrusted, thick-, or thin-walled interwoven hyphae (e), middle textura angularis (g), and innermost textura subglobolosa to late ellipsoidalis (h).

450 × 270–410 μm, maturescentia intra 2 menses. Paries gymnothecia, circumcinctus per et laxum, intertextum, septatum, crassitunicatum, verrucosum et levem, tenuitunicatum hypham, textura constituta ex compressis arte implexis, aliquantum dissimilibus hyphis, simulans ex parenchymatis texturis. Initia constantia ex aseptata, laeves, hyaline, afflata, curvata vel circinata hyphis intium e intercalari ex verrucoso vel levi hypha, quae septata, ramosa, profusa, ac evoluta in ascogenoam hypham. Nec antheridia nec ascogonia observata. Asci evanescentes, globosi, subglobosi, hyalini, in breves catenas, octospori, 8.5–10.8 × 7.3–9.2 μm. Ascospora ellipsoidea, hyalina, subhyalina, pallida flava in massa, 3.3–6.7 × 2.8–4.2 μm, maxime aspera ad spinosa, vulgo cum monoaequatoria crista, usque ad 1.3 μm latae, minus saepe crista irregulariter vel intermittim disposita, in Y-, X-, spirali-, cruciata. Conidiophora portata e funiculosis vel aeriis hyphis. Stipes erectus, strictus, flexuous vel sinuosus, hyalinus, septatus, fere subtiliter asper ad asperum, aliquando lenem, 35–180 × 2.6–4.2 μm. Penicillum monoverticillatum, biverticillatum vel asymmetrice irregulare. Metula appressa, in verticillos ex 2–5, subtiliter asperis ad asperas, raro laeves, 8.3–27.4(54) × 2.1–3.3 μm. Phialis fere 2–5 per metulam, vel 3–7 per stipitem in stata ex monoverticillato, acerosa, raro ampulliformis, laevis ad subtiliter asperam, 6.7–22 × 2.1–3 μm, multum cum longa gradatim attenuata collulo, usque ad 7.1 μm in longitudine. Conidia fere ellipsoidea, ovoidea, minus fere subglobosa vel pyriformia, hyalina, glabro-tunicata, 2.7–5 × 1.7–3.2 μm, portata in implicatis, inordinatis catenis.

HOLOTYPUS ex solo, Funlu, Chiayi Comitato, Taiwan, R.O.C. PPH16, ISOTYPUS IMI; New York Botanicus Hortus.

SPECIMEN EXAMINED. Isolated from soil heated at 60 C for 20 min, from Funlu, Chiayi County, Taiwan, R.O.C., June 28, 1990. HOLOTYPE PPH16 (dried culture) derived from PPH16E (living culture) was deposited in the Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, R.O.C. PPH16E was also deposited in the Culture Collection and Research Center (CCRC 32703), Hsinchu, Taiwan, R.O.C. ISOTYPES in NY and in IMI.

On CYA, 25 C, 7 days, colony growing restrictedly, 10.5–16 mm diam (FIG. 4), dense, raised, floccose, white to reddish grey (10–11B2); mycelium white to reddish grey (10–11B2); conidiogenesis not observed on CYA; soluble pigment pale greyish, pale red to reddish brown (8C–D5, 9D6); exudate absent; reverse reddish white, reddish orange, greyish red to reddish brown or violet brown (7A2–6, 7B4–6, 8B–D4–6, 10–11E7–8).

On MEA, 25 C, 7 days, colony 15–19.5 mm diam (FIG. 5), dense, floccose, villose or funiculose; margin lower, fibrillous; mycelium white to yellowish white (3–4A2); hyphae branched, septate, most commonly smooth or occasionally roughened, hyaline to subhyaline, pale yellow brown in mass, 1–4.2 μm diam; ascomata discrete or occasionally confluent; suspended on the aerial hyphae or immersed, enmeshed within the mycelial matrix; conidiogenesis moderate, white; soluble pigment pale greyish red; exudate, clear to pale yellow; reverse pastel (light) yellow, yellow (3A4–6, 5A–B6–7) or light orange becoming dark orange (6A4–8, 6B7–8) to reddish orange (7A–B6) in age.

On GN25N at 25 C for 7 days, no growth.

On OMA, 25 C, 7 days, colony, 23–28 mm diam, thin and flat, center somewhat floccose, submargin to margin velvety, white; mycelium white; hyphae branched, septate, most commonly smooth occasionally roughened, hyaline to subhyaline, pale yellow brown in mass, 0.8–5.5 μm diam; conidiogenesis conspicuous; conidiophores mostly ascending from substratum; conidia mostly ovoid, smooth or nearly so, hyaline, white in mass; reverse white to yellowish white

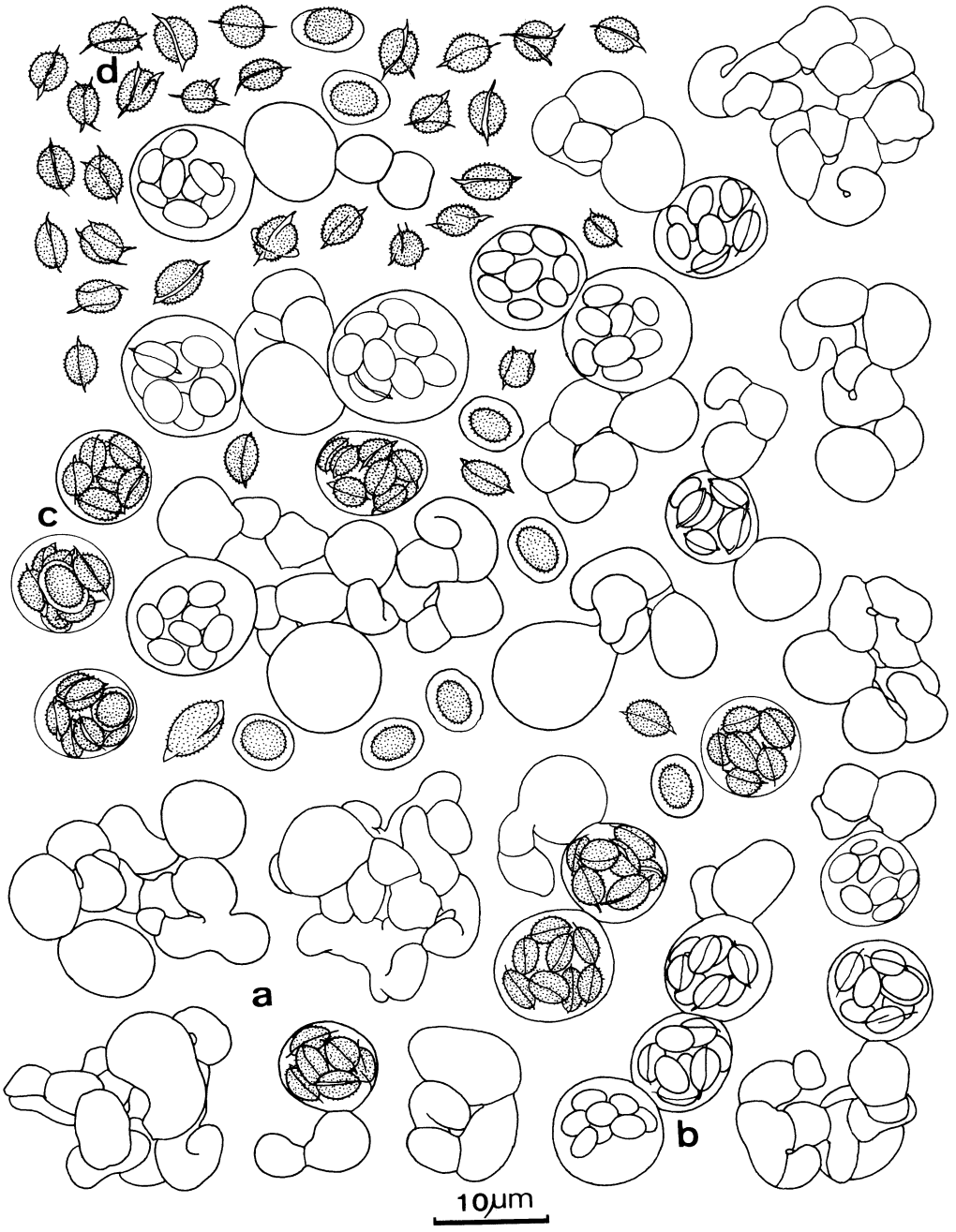


FIG. 2. *Talaromyces unicus*. a. Ascus primordia. b. Asci in short chain. c. Asci. d. Ascospores spinose, with monoequatorial or irregularly disposed X-, Y-, spiral-, or cross-shaped flanges.

(4A2); gymnothecia usually lacking, but occasionally formed in the sector in some colonies, and ripening approximately in 4 wk; exudate and soluble pigment absent.

On CYA at 5 C or 37 C, 7 days, no growth.

Gymnothecia soft, abundant on MEA, usually discrete, occasionally confluent, globose, subglobose, or ellipsoidal (FIGS. 14, 15), pastel yellow

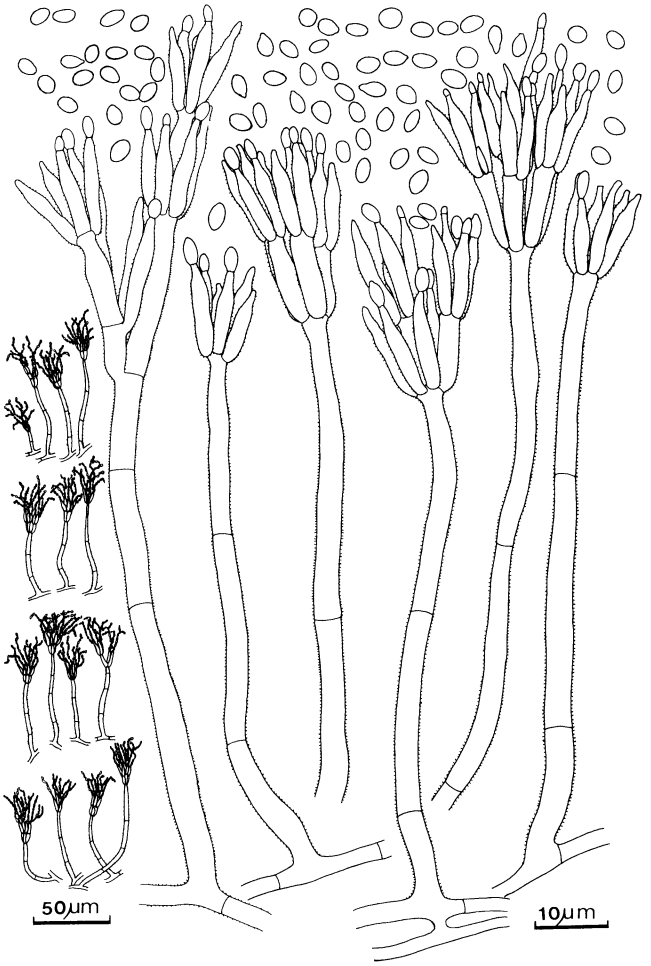
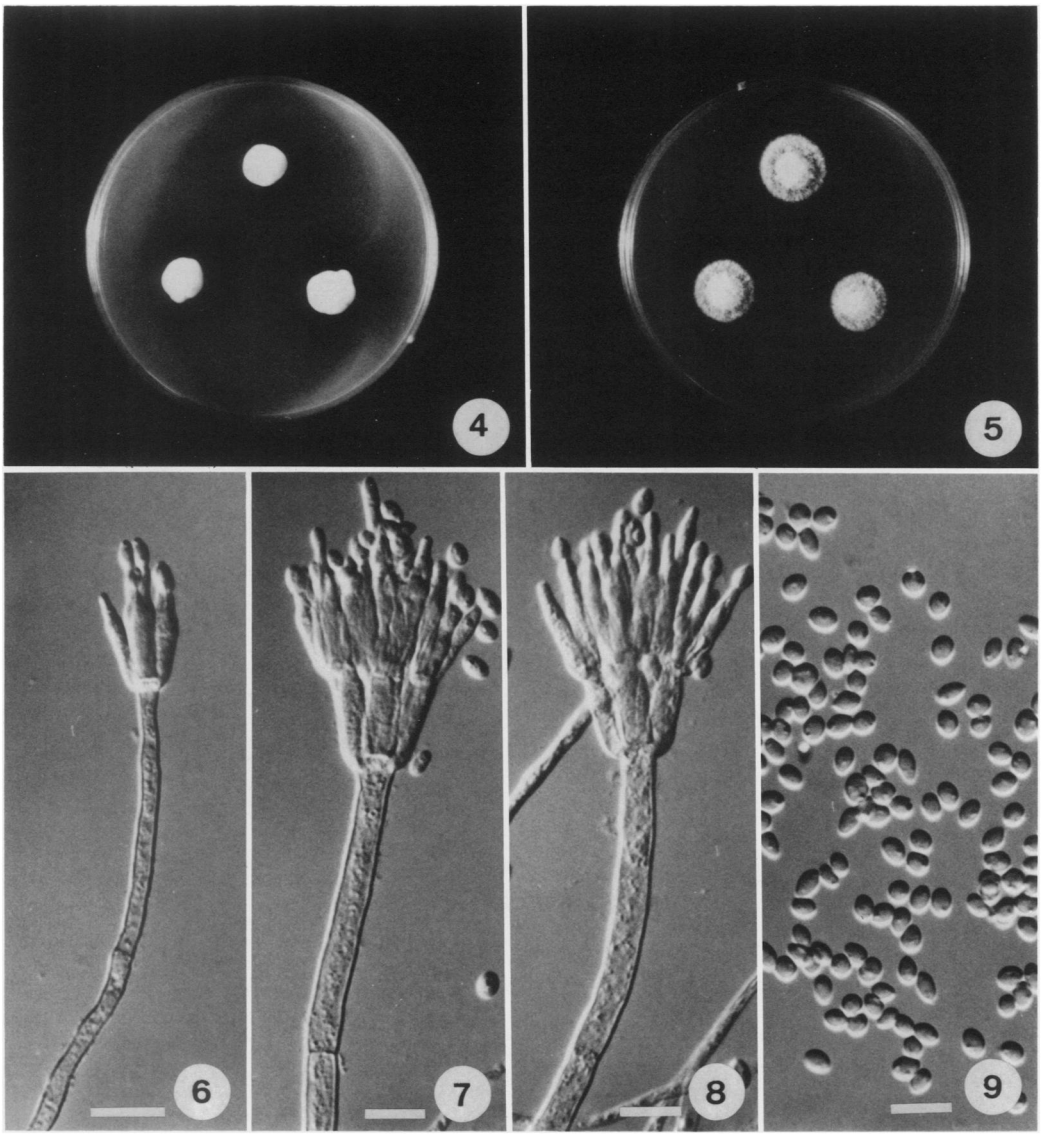


FIG. 3. *Talaromyces unicus* (= *Penicillium unicum*). Penicilli and conidia.

or yellow (3A4–6), 280–450 × 270–410 μm, maturing mostly in 2 months. Ascocarps surrounded by loose weft of interwoven, septate, thick-walled verrucose, and the smooth, thin-walled hyphae (FIG. 1e). Ascocarp peridium (FIG. 1f–h) composed of three layers of compressed closely knit somewhat differentiated hyphae simulating a pseudoparenchymatous tissue (FIGS. 15, 16); the outermost textura epidermoidea (FIG. 1f), middle textura angularis (FIG. 1g), innermost textura subglobulosa to late ellipsoidalis (FIG. 1h). Ascogonial initials consisting of aseptate, smooth, hyaline, swollen, curved or coiled cells intercalarily initiated from the verrucose mycelium, septating, branching profusely, and finally developing into ascogenous hyphae (FIG. 1a, b). Neither antheridia nor ascogonia were observed. Asci evanescent, globose, subglobose, hyaline, subhyaline, pale yellow in mass, in slightly curved short chains, eight-spored, 8.5–10.8 × 7.3–9.2 μm (FIG. 2). Ascospores ellipsoidal, hyaline, 3.3–6.7 × 2.8–4.2 μm, very roughened to spinose, usually with a single flange, up to 1.3 μm wide, less often the flange is irregularly or discontinuously disposed, in Y-, X-, spiral-, or cross-shapes (FIGS. 2, 17–28).

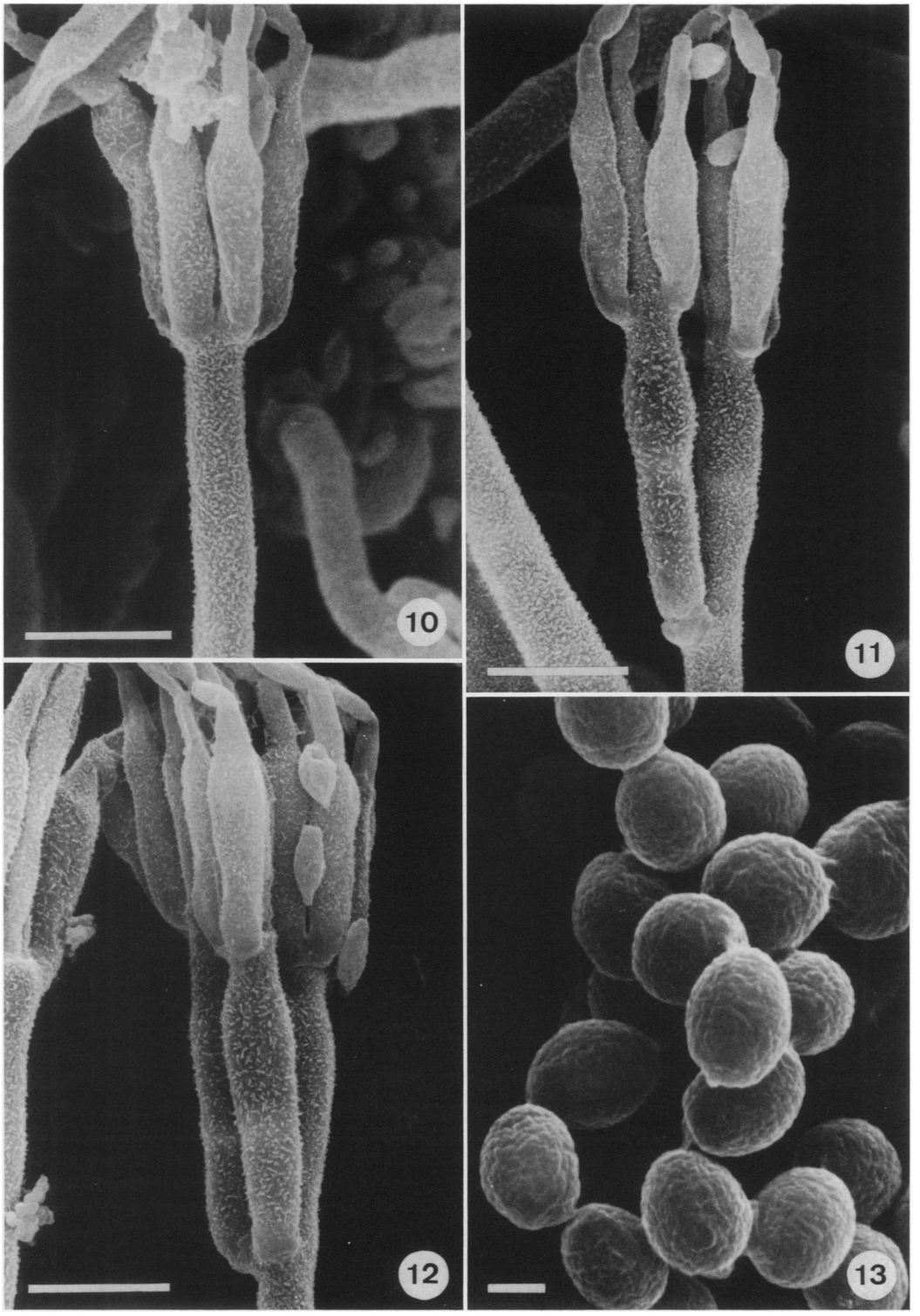
Conidiophores on MEA arising from funicules or aerial hyphae. Stipes erect, straight, flexuous or sinuous, hyaline, septate, mostly finely roughened to roughened, occasionally smooth, 35–180 × 2.6–4.2 μm. Penicilli monoverticillate, biverticillate, symmetric or asymmetric (FIGS. 3, 6–8). Metulae appressed, in verticils of two to five, finely roughened to roughened (FIGS. 10–12), oc-



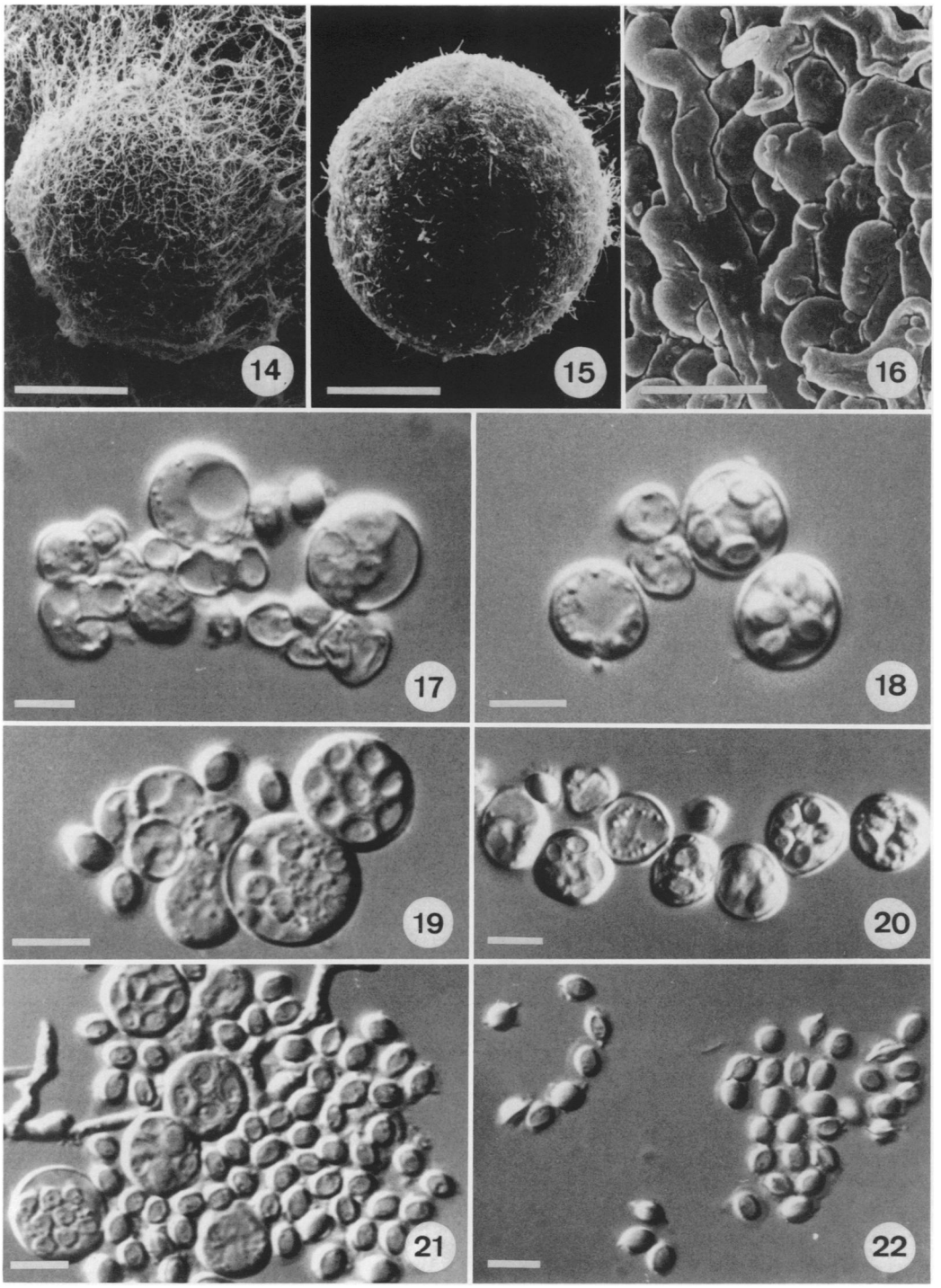
FIGS. 4–9. *Talaromyces unicus* (= *Penicillium unicum*). 4, 5. Colonies on CYA and MEA for 7 days at 25 C. 6. Monoverticillate penicillus. 7, 8. Biverticillate penicillus with lanceolate phialides. 9. Ellipsoidal conidia under light microscopy. FIGS. 4, 5, ×0.5; FIGS. 6–9, bar = 5 μm.

casionally smooth, 8.3–27.4(54) × 2.1–3.3 μm; phialides commonly 2–5 per metula, or 3–7 per stipe in monoverticillate penicilli, acerose, rarely ampulliform (FIGS. 3, 6–8, 10–12), smooth to finely roughened, 6.7–22 × 2.1–3 μm, often with long, gradually tapering collula, up 7.1 μm in length (FIGS. 10–12). Conidia mostly ellipsoidal, ovoid, less commonly subglobose or pyriform, hyaline, smooth-walled, 2.7–5 × 1.7–3.2 μm, borne in tangled, disordered chains (FIGS. 9, 13).

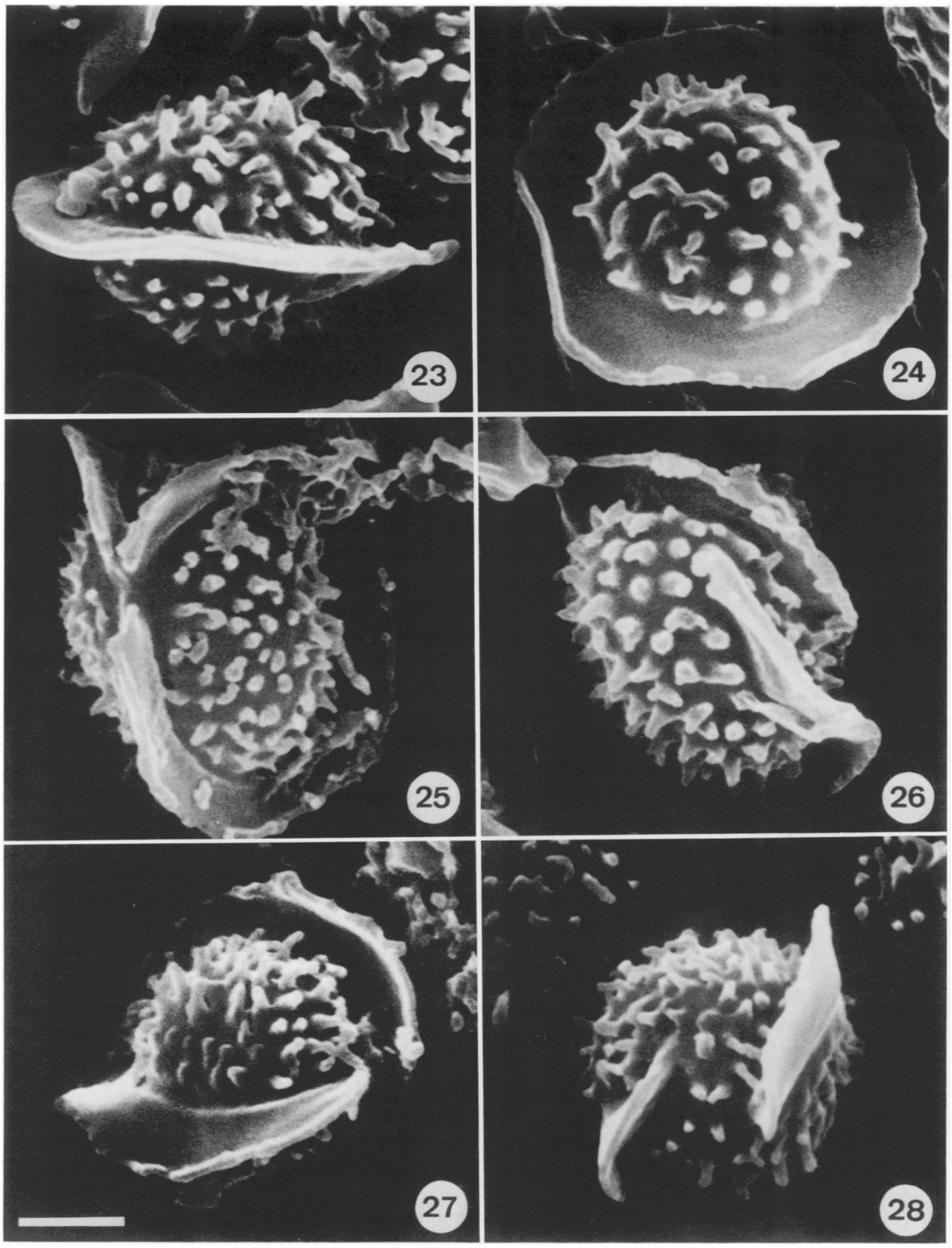
Talaromyces unicus failed to grow at 5 C and at 37 C. It is therefore mesophilic, despite the fact that heat may have stimulated the dormant ascospores to germinate and caused it to be more easily isolated on selective media like DRBC. It has been encountered only once in a 2-yr survey, and so it is a rare species with low occurrence



FIGS. 10–13. *Talaromyces unicus* (= *Penicillium unicum*). Scanning electron micrographs of penicilli and conidia. Penicilli mono- (FIG. 10), or biverticillate (FIGS. 11, 12), phialides, metulae, and stipes finely roughened (FIGS. 10–12). Conidia somewhat wrinkled (FIG. 13). FIGS. 10–12, bar = 5 μm; FIG. 13, bar = 1 μm.



FIGS. 14–22. *Talaromyces unicus.* Gymnothecia, ascus and ascospores. 14. Gymnothecium surrounded by loose weft of interwoven hyphae. 15. Soft gymnothecium with wall simulating pseudoparenchymatous tissue. 16. An enlarged view of the outermost layer of the gymnothecial wall with textura epidermoidea. 17–21. Asci globose, or subglobose (FIGS. 17–19), in chain (FIG. 20), with 8 ascospores each (FIG. 19), evanescent (FIG. 21). 22. Ascospores with mono- or irregular flanges. FIGS. 14, 15, bar = 100 μm; FIGS. 16–22, bar = 5 μm.



FIGS. 23–28. *Talaromyces unicus*. Scanning electron micrographs of ascospores. 23. Lateral view of ascospore, showing the uniequatorial flange and spinose ornamentation. 24. Top view of ascospore, showing entire, uniequatorial flange. 25. Ascospore with Y-shaped flange. 26. Ascospore with discontinuous, spiral-like flange. 27. Ascospore with cross-shaped flange. 28. Ascospore with discontinuous, somewhat parallel flange. Bar in FIG. 27 for FIGS. 23–28 = 1 μm.

and restricted distribution. On MEA medium gymnothecia ripen in approximately 2 months, apparently longer than most described *Talaromyces* spp. (Stolk and Samson, **1972**; Pitt, **1979**). Gymnothecia also can be obtained from a single spore culture which indicates *T. unicus* is probably homothallic. Its striking ascospore ornamentation and peculiar disposition of the flanges make it readily distinguishable from other *Talaromyces* spp. (Pitt, **1979**; Stolk and Samson, **1971**, **1972**, **1983**; Samson and Mahoney, **1977**; Samson and Abdel-Fattah, **1978**).

The ascomatal coverings of *T. unicus* are distinct, yellow, closely knit, somewhat simulating a pseudoparenchymatous wall. Ascomatal initials develop as branches or as intercalary portions of the hyphae. Asci are formed in short chains, with eight ascospores, ellipsoidal or ovoid, subhyaline, and their wall showing various ornamentations. The penicilli of *T. unicus* (= *Penicillium unicum*) are monoverticillate, or biverticillate, metulae bearing lanceolate phialides, mostly hyaline, finely roughened to roughened; conidia are hyaline, smooth under light microscopy but somewhat wrinkled under scanning electron microscopy. Apparently, most of these characteristics of *T. unicus* are in agreement with *Talaromyces* section *Talaromyces,* though with a minor deviation. After an appraisal of these characters, *T. unicus* herein is designated as a member of *Talaromyces* section *Talaromyces* (Stolk and Samson, **1972**).

T. unicus is more closely related to *T. luteus* (Sacc.) Stolk & Samson, *T. stipitatus* Benjamin, *T. ohiensis* Pitt, *T. udagawae* Stolk & Samson (*Talaromyces* section *Talaromyces*), *T. leycettanus* Evan & Stolk (*Talaromyces* section *Emersonia*), *T. thermophilus* Stolk (*Talaromyces* section *Thermophila*) in terms of the morphological characteristics of the holomorph. However, it can be distinguished from these species by some microscopic features.

Ascomata of *T. luteus* are yellow, ripening within 2–3 wk, the covering consisting of a well-developed network of interwoven hyphae. Ascogonial initials develop usually from atypical phialides, occasionally from hyphae. Ascospores are ellipsoidal, 4.5–5.5 × 3–3.7 μm, with four to six conspicuous, irregularly transverse, locally interrupted, occasionally branched, 0.5 μm ridges. Conidiophores are smooth, occasionally encrusted, usually 15–35 μm, occasionally up to 100 μm in length. Phialides are lanceolate, apices in old structures occasionally greenish. Conidia ovoid to ellipsoidal, smooth, green in mass (Stolk and Samson, **1972**). *T. unicus* is similar to *T. luteus* in having yellow ascomata, but it takes longer time (ca 2 months) for ripening; also the coverings are much more distinct, closely knit, simulating a pseudoparenchymatous wall. In contrast to *T. luteus,* ascospores of *T. unicus* are very roughened to spinose, usually with a single equatorial flange, though irregularly transverse, locally interrupted or cross, up to 1.3 μm wide flanges are less often present (FIGS. 22–28). The finely roughened conidiophores (FIGS. 10–12) of *T. unicus* usually are longer than in *T. luteus,* though the verticilation of penicilli are similar. The size and shape of conidia of both species are similar but distinct in color; *T. luteus* are green in mass, whereas *T. unicus* are hyaline to subhyaline, pale yellow in mass.

Ascomata of *T. stipitatus* are usually yellow, in some strains pinkish to salmon-colored, ripening rapidly (10–14 days), coverings distinct, simulating a pseudoparenchymatous wall. Initials consist of ascogonia and antheridia. Ascospores are yellow, flattened, ellipsoidal, with a single equatorial, 0.2–0.5 μm ridge, smooth-walled. Phialides are lanceolate with a long neck. Conidia are ellipsoidal, ovoid to pyriform, hyaline to pale greenish, smooth-walled (Stolk and Samson, **1972**). The color and covering of *T. unicus* resemble those of *T. stipitatus,* but it lacks gametangia, its ascogonial initials develop directly from intercalary swollen, curved or coiled cells from the verrucose hyphae (FIG. 1a–d). Though ascospores of *T. unicus* often have a single equatorial flange similar to *T. stipitatus,* the roughened, spinose, or less often irregular disposition of flanges (FIGS. 23–28) readily distinguish these two species.

Gymnothecia of *T. ohiensis* are composed of closely interwoven hyphae, dull yellow, maturing in 2–3 wk, initials convolute, enlarging and branching to form ascogenous hyphae, ascospores yellow, ornamented with fine striations, often discontinuous, commonly longitudinal but also oblique or sometime transverse. Penicilli are biverticillate, infrequently, monoverticillate; conidia ellipsoidal, smooth to finely roughened (Pitt, **1979**). *T. ohiensis* has ascomata color, ascogonial ontogenesis, penicilli, and conidial morphology similar to *T. unicus* but can be rapidly separated

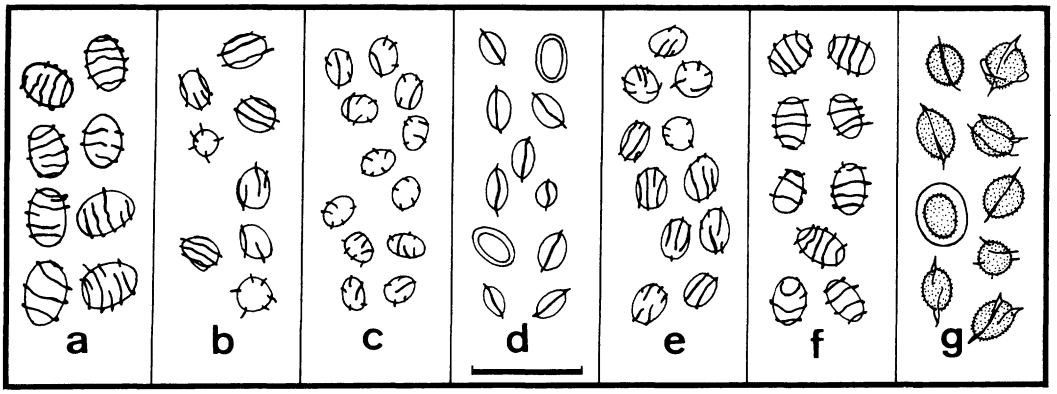


FIG. 29. A synoptic illustration of the ascospore ornamentation of some *Talaromyces* spp. *T. luteus* (a), *T. leycettanus* (b), *T. ohiensis* (c), *T. stipitatus* (d), *T. thermophilus* (e), *T. udagawae* (f), and *T. unicus* (g). Bar = 10 μm. a–f. Courtesy of Stolk and Samson (**1972**).

by the length of the conidiophores and phialides, ascomata coverings (FIG. 1f–h), ascospore color and ornamentation (FIGS. 23–28).

Ascomata of *T. udagawae* are yellow to orange, the covering consisting of thin networks of loosely interwoven hyphae. Ascogonial initials develop from terminal or intercalary swellings and branching of hyphae. Ascospores are ellipsoidal, ornamented with three to five regularly transverse, nearly parallel ridges, often spirally arranged. Phialides are lanceolate, 7.5–10 × 2.2–4 μm. Conidia are hyaline, subglobose to ellipsoidal, smooth-walled (Stolk and Samson, **1972**). Apparently, *T. unicus* has more elongate phialides (6.7–22 × 2.1–3.0 μm); also it has varied, ornamented ascospores (FIGS. 2, 22–28) different from *T. udagawae*.

T. leycettanus is classified in *Talaromyces* section *Emersonii*. Ascomata of *T. leycettanus* are greenish-yellow, the covering consisting of thin hyphal networks. Ascogonial initials consist of coiled hyphae. Ascospores are ellipsoidal, ornamented with two to six thin, somewhat jagged, irregular, mostly longitudinal ridges of different lengths. The anamorph of *T. leycettanus* is *Paecilomyces*; conidia are yellowish, yellow-brown in mass, cylindrical, chlamydospores thick-walled, globose or nearly so, yellowish. The species is thermotolerant to thermophilic (Stolk and Samson, **1972**). The differences between *T. unicus* and *T. leycettanus* are distinct. *T. unicus* is mesophilic, with *Penicillium* anamorph, ascomata having a pseudoparenchymatous covering; also ascospore ornamentation is varied (FIGS. 23–28).

T. thermophilus is strongly thermophilic and is classified in *Talaromyces* section *Thermophila*. Ascomata of *T. thermophilus* have well-developed, thick, parenchymatous walls. Ascogonial initials consist of irregularly swollen cells which coil and branch thus forming a compact mass of ascogenous hyphae. Ascospores are ellipsoidal, ornamented by two to six somewhat jagged, irregular, usually longitudinal ridges. Conidiophores mostly arise as short or less perpendicular branches from aerial hyphae; Penicilli are irregular; rami and metulae elements are somewhat divergently arranged; phialides usually consist of a cylindrical basal portion which tapers abruptly to a distinct neck about 1.5 μm in length; conidia are ellipsoidal; chlamydospores are abundant, thick-walled (Stolk and Samson, **1972**). As a result of overall comparison of physiological characters and gross morphology in rami, metulae, phialide, chlamydospores and ascospore ornamentation, *T. thermophilus* and *T. unicus* can be readily separated (see FIGS. 23–28).

To avoid confusion and for clear comparisons, a synoptic illustration (FIG. 29) of ascospores of *T. unicus* and closely related *Talaromyces* spp. is provided.

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