

# Taxonomic studies on new or critical fungi of non-pathogenic Onygenales 3

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**Two new species of soil-borne onygenalean fungi are described and illustrated: *Arachniotus insolitus*, isolated from forest soil in Kenya, and *Myxotrichum stellatum*, isolated from forest soil in Japan. A further occurrence of *Gymnoascus uncinatus*, isolated from Canadian soil, is reported and some observations are added, especially in connection with the cultural appearance of this rarely encountered species.**

Key Words—ascmycetes; Onygenales; soil fungi; systematics.

Previous papers (Udagawa and Uchiyama, 1999a, b) reported a number of new onygenalean taxa isolated from soils in Japan and overseas. Meanwhile, three unusual isolates have been obtained from additional soil samples. One was identified as *Gymnoascus uncinatus* Eidam, a rarely encountered fungus, and the other two were found to be different from all described species of *Arachniotus* J. Schroet. and *Myxotrichum* Kunze.

## Taxonomy

*Arachniotus insolitus* Udagawa et Uchiyama, sp. nov. Figs. 1–9

Coloniae in agaro farinae avenaceae mixto (OA) celeriter crescentes, floccosae, ex mycelio basali coacto tenuiter constantes, ascomatibus abundantibus formantes, primo albae, deinde rubrae vel persicinae; conidiogenesis limitata; reversum griseo-aurantiacum vel melleum.

Ascomata saepe confluentia, globosa vel subglobosa, appendiculis inclusis 250–850  $\mu\text{m}$  diam, rubro-aurantiaca vel rubro-brunnea. Hyphae peridii rubro-brunneae, incrassatae, leves vel asperulatae, septatae, 4–8  $\mu\text{m}$  diam, ramosae et anastomosantes, completo-reticulo formantes, ad extremum truncatae vel appendicibus brevibus formantes; appendices spiniformes, 20–75  $\times$  4–6  $\mu\text{m}$ , asperulatae, rubro-brunneae, superne pallescens, ad apicem obtusae vel rotundatae vel parum subacutae, interdum bi- vel trifurcatae. Asci 8-spori, subglobosi vel ovoidei vel pyriformes, 13–15  $\times$  10.5–13  $\mu\text{m}$ , evanescentes. Ascospores flavo-brunneae, oblatas, apice truncatae, saepe cum sulco aequatorio vadoso instructae, 5–6.5(–7)  $\times$  3–4(–5)  $\mu\text{m}$ , verruculosae vel foveolatae.

Mycelio vegetativo ex hyphis hyalinis, ramosis, levi-

bus, septatis, 1–4  $\mu\text{m}$  diam, saepe fasciculatis composito. Arthroconidia et aleurioconidia producentia.

Holotypus: SUM 3138; colonia exsiccata in cultura ex solo sylvae, in Kenya, 24.III.1996, a S. Uchiyama isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis Chiba (Natural History Museum and Institute, Chiba, Japan; CBM) conservata.

Etymology: Latin, *insolitus*=unusual, referring to the characteristics of the ascomatal peridium, which are not consistent with those of the type species of the genus.

Colonies on OA growing rapidly, attaining a diam of 30–32 mm in 14 d at 25°C, floccose, consisting of a thin basal felt, producing abundant ascomata and limited conidia in the aerial tomentum, at first white, later becoming Pastel Red (M. 8A4, after Kornerup and Wanscher, 1978) or Peach (Rayner, 1970); margins thin; exudate small, clear; reverse Greyish Orange (M. 5B3) or Honey (R). Colonies on cellulose agar growing slowly, attaining a diam of 35–36 mm in 28 d at 25°C, plane, with vegetative mycelium submerged, consisting of a thin layer of ascomata adjacent to the agar surface, overgrown by a loose network of aerial hyphae, in color as on OA; conidia few in number, not affecting the colony appearance; reverse uncolored. Colonies on PYE growing more rapidly than above, 42–44 mm in 14 d at 25°C, more or less floccose, radially sulcate, consisting of a close-textured mycelial felt; ascomata slowly developed, Pale Orange (M. 6A3) or Rosy Buff (R); conidiogenesis indistinct; reverse Light Orange (M. 5A4) or Pale Luteous (R).

Ascomata discrete or often confluent, globose to subglobose, 250–850  $\mu\text{m}$  in diam incl. appendages, initially white, becoming reddish orange to reddish brown, maturing within 28 d; centrum 160–420  $\mu\text{m}$  in diam, light yellow to orange red. Peridial hyphae reddish brown, thick-walled, smooth to asperulate, septate, 4–

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8  $\mu\text{m}$  in diam, usually not constricted or swollen at the septum, branched and anastomosed, forming a reticulate network, with free apices truncate or forming short appendages; appendages spine-like or boat-hook-shaped, 20–75  $\times$  4–6  $\mu\text{m}$ , asperulate, reddish brown, paling above, with apices blunt, rounded or slightly subacute, sometimes bifurcate or trifurcate with the branches in different planes. Asci 8-spored, subglobose to ovoid or pyriform, 13–15  $\times$  10.5–13  $\mu\text{m}$ , evanescent. Ascospores yellowish brown, oblate with truncate apices, often with a shallow equatorial furrow and bipolar wall thickenings, 5–6.5(–7)  $\times$  3–4(–5)  $\mu\text{m}$ , with surface verruculose to slightly pitted.

Vegetative mycelium consisting of hyaline, branched, smooth-walled, septate, 1–4  $\mu\text{m}$  diam hyphae which are often bundled; racquet hyphae present; ascumatal initials arising from two adjacent cells of the same hypha or separate hyphae and becoming closely appressed to one another to give a mutually coiling initial.

Anamorph: arthroconidia, which are hyaline, cylin-

drical, 8–20  $\times$  2.5–4  $\mu\text{m}$ , truncate at both ends, smooth or finely roughened, and aleurioconidia, which are terminal, hyaline, pyriform, 8–10  $\times$  4–5  $\mu\text{m}$ , truncate at the base, smooth or asperulate.

Weakly keratinolytic as well as cellulolytic.

At 37°C, growth is nil.

Holotype: SUM 3138, a dried culture isolated from forest soil, Kirinyaga (alt. 3,200 m), Mt. Kenya, Kenya, 24 March 1996, col. S. Uchiyama (CBM).

Other specimens examined for comparison: *Arachniotus ruber* (van Tiegh.) J. Schroet. strain ATCC 15315, isolated from soil, U. K., 1961, by J. Cox, and strain ATCC 16945, isolated from wheat-field soil, Germany, by K. H. Domsch (Fig. 10); *Gymnoascus corniculatus* G. F. Orr et Plunkett strains NRRL A-10098 (Figs. 11, 12) and A-10099, isolated from soil, U.S.A., by G. F. Orr.

Note: The placement of *A. insolitus* within the genus *Arachniotus* in the Gymnoascaceae poses a difficult problem. The morphological characteristics of its ma-

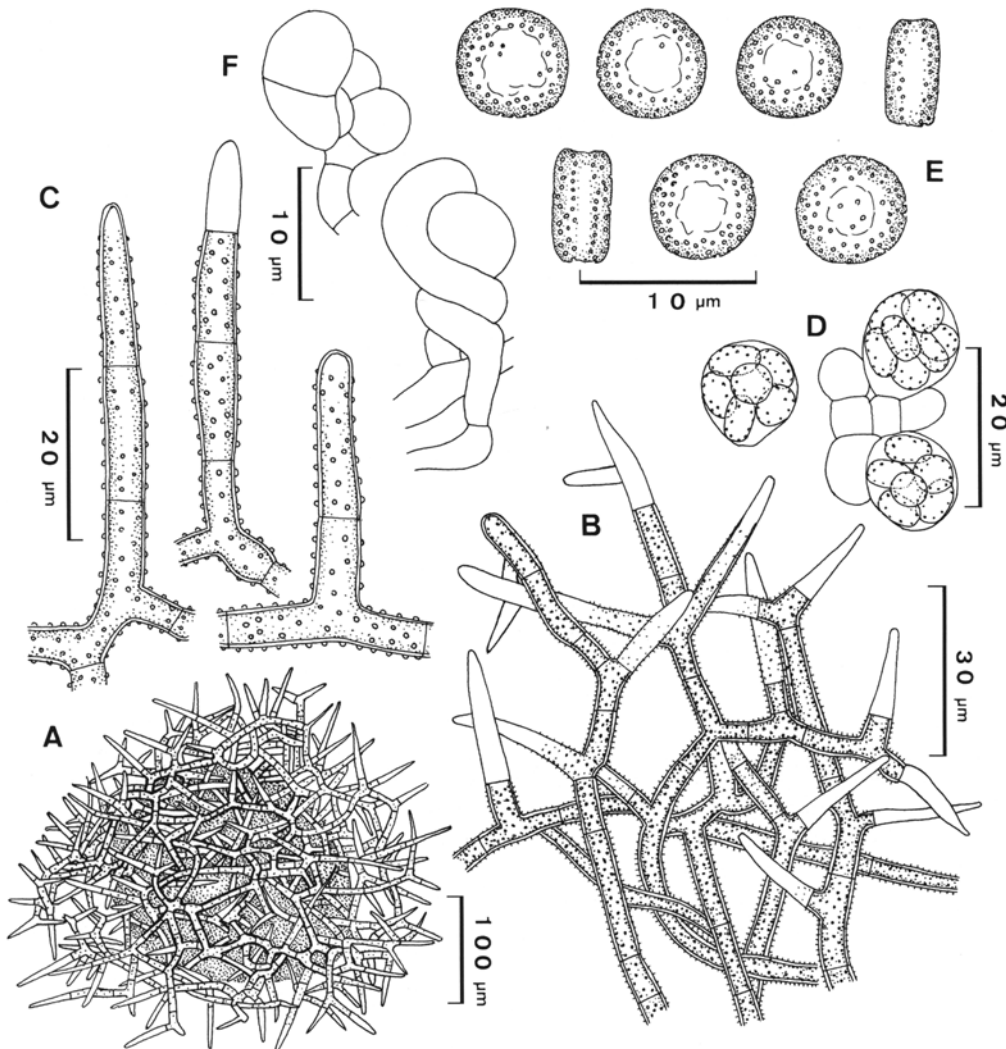


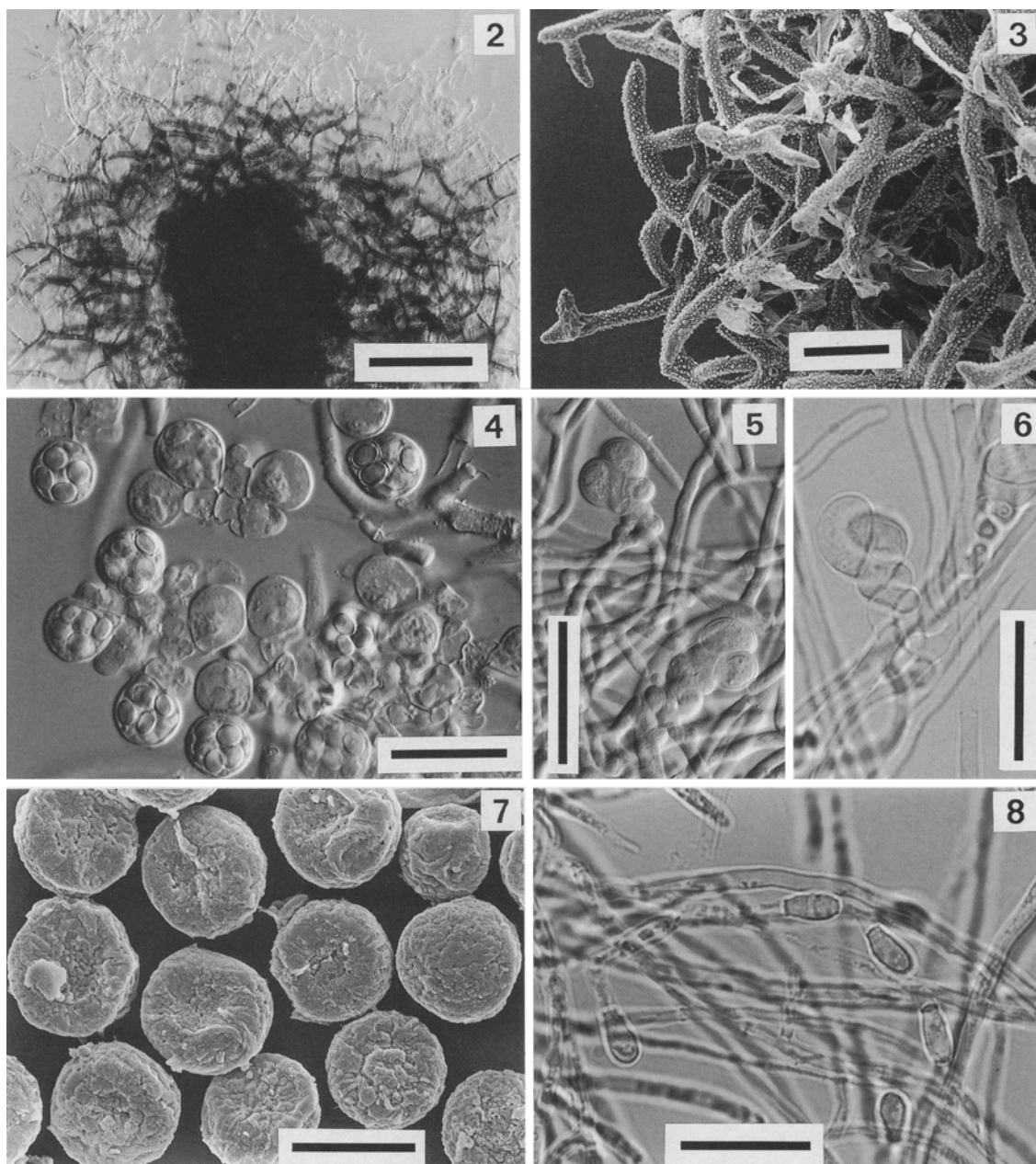
Fig. 1. *Arachniotus insolitus* (SUM 3138).

A. Ascoma. B. Margin of ascoma showing appendages. C. Simple apices of peridial hyphae. D. Asci. E. Ascospores. F. Ascumatal initials.

ture ascospores (color, shape, size, surface ornamentation, etc.) are strongly suggestive of the genus *Uncinocarpus* Sigler et G. F. Orr (Sigler and Carmichael, 1976). *Uncinocarpus* was recently reviewed by Sigler et al. (1998); three species, *U. reesii* Sigler et G. F. Orr, *U. orissi* (B. Sur et G. R. Ghosh) Sigler et Flis, and *U. queenslandicus* (Apinis et R. G. Rees) Sigler, were accepted under the emended generic concept. These species are united in this genus on the basis of (1) globose gymnothecial ascomata without differentiated ascumatal hyphae, (2) oblate yellowish brown or reddish

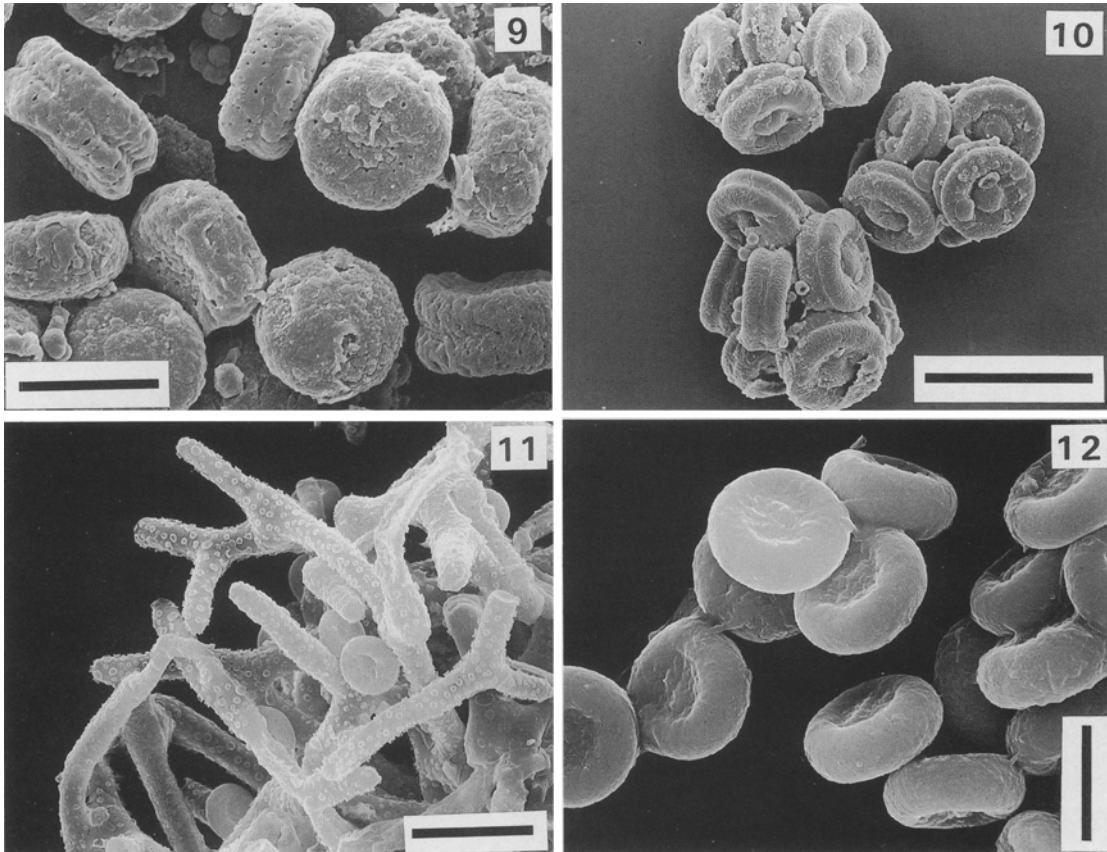
brown ascospores ornamented with puncta, (3) bulbous ascumatal initials, (4) anamorph characterized by clavate terminal aleurioconidia and (or) intercalary arthroconidia, (5) capacity to digest hairs in vitro and produce perforating bodies, and (6) thermotolerance. However, this fungus is quite different from *Uncinocarpus* species in a number of features: e.g., ascumata with anastomosing elements forming a reticuloperidium, mutually coiling initials of the ascumata, and mesophilic nature and inability to grow at 37°C.

The general morphology of its ascumata is also sug-



Figs. 2–8. *Arachniotus insolitus*.

2. Ascoma. 3. Appendages (SEM). 4. Asci. 5, 6. Ascumatal initials. 7. Ascospores (SEM). 8. Terminal and intercalary conidia. Scale bars: 2 = 100  $\mu\text{m}$ ; 3–6, 8 = 20  $\mu\text{m}$ ; 7 = 5  $\mu\text{m}$ .



Figs. 9–12. *Arachniotus insolitus*, *Arachniotus ruber* (ATCC 16945) and *Gymnoascus corniculatus* (NRRL A-10098).

9. Ascospores of *A. insolitus*, showing a shallow equatorial furrow and bipolar wall thickenings (SEM). 10. Ascospores of *A. ruber* (SEM). 11. Appendages of *G. corniculatus* (SEM). 12. Ascospores of *G. corniculatus* (SEM). Scale bars: 9, 12 = 5  $\mu\text{m}$ ; 10, 11 = 10  $\mu\text{m}$ .

gestive of the genus *Gymnoascus* Baran. It shows some resemblance to *Gymnoascus corniculatus* G. F. Orr et Plunkett as described by Orr et al. (1963a). According to the description, the habits of growth, coloration, peridial structure and ascospores of *G. corniculatus* duplicate those of the type species, *Gymnoascus reessii* Baran. The single major criterion separating *G. corniculatus* from *G. reessii* is the trifurcate mode (Fig. 11) of branching at the apex of approximately 50% of the ascumatal appendages. Such trifurcations are also present in this fungus; however, it differs decisively from the *Gymnoascus* species in the ascospore characteristics, which are distinctive in being pulley-form with truncate apices, a shallow equatorial furrow and only slightly pitted surface in contrast with oblate but marginally narrowed, smooth-walled ascospores (Fig. 12).

The ascospores of this fungus clearly align the species with *Arachniotus ruber*, the type species of the genus (Fig. 10). The outstanding characteristics of *A. ruber* are: (1) orange to red, globose ascumata with a loose envelope of thin-walled hyphae scarcely differentiated from the vegetative hyphae, (2) ascumatal initials composed of two similar gametangia that coil about each other forming a double helix, (3) ascospores that are

orange to yellow, oblate, pulley-form with a shallow equatorial furrow and polar thickenings, smooth-walled, (4) unable to grow at 37°C, and (5) cellulolytic ability (Apinis, 1964; Kuehn and Orr, 1964; Domsch et al., 1980; Currah, 1985). The anamorph of *A. ruber* has been stated to have hyaline arthroconidia (Apinis, 1964; Kuehn and Orr, 1964), but Currah (1985) in his study of the neotype strain and some additional materials was unable to find conidial production. Thus the case for inclusion of this fungus in *Arachniotus* would require broadening of the generic concept, because its ascumatal peridium is composed of thick-walled, markedly differentiated hyphae which form a distinctive reticulum and its slightly pitted ascospore ornamentation. On the basis of ascospore shape and the combination of other general features including colony coloration, ascumatal initials and growth temperatures, this fungus is regarded as currently assignable in the genus *Arachniotus*, but somewhat transitional in the direction of *Gymnoascus*. For the determination of the phylogenetic relationships of *Arachniotus* and *Gymnoascus*, however, future studies such as DNA sequencing are desired.

***Myxotrichum stellatum*** Udagawa et Uchiyama, sp. nov. Figs. 13–20

Coloniae in agar decocto tuberorum (PDA) dilute tarde crescentes, velutinae, plicatae vel rugosae, ex coacto mycelio compacto constantes, atrovirides vel olivaceo-nigrae vel ferro-griseae, abundantibus ascomatibus formantes; reversum atroviride vel viridi-griseum vel viridi-nigrum.

Ascomata saepe confluentia, plus minusve globosa, appendiculis inclusis 200–300 (–500)  $\mu\text{m}$  diam, brunneo-grisea. Hyphae peridii hyalinae vel atrobrunneae, incrassatae, septatae, 1.5–3  $\mu\text{m}$  diam, leves vel asperulatae, ramosae et anastomosantes, reticulo laxo formantes; appendices spiniformes, dilute brunneae, rectae vel leviter flexae, breves, 12–40  $\times$  1.5  $\mu\text{m}$ , vulgo non ramosae, septatae, incrassatae, asperulatae, apice hyalinae et acutae vel obtuse rotundatae. Asci 8-spori, ovoidei vel late clavati vel pyriformes, 14–23  $\times$  8–12  $\mu\text{m}$ , brevistipitati, evanescentes. Ascosporae dilute flavae vel flavo-brunneae, fusiformes vel ellipsoideae, 4–6  $\times$  3–3.5  $\mu\text{m}$ , superne 5–6 longitudinalibus striis striatae, in sectione transversali stellatae. Mycelio vegetativo ex hyphis hyalinis, ramosis, septatis, levibus, 1–3  $\mu\text{m}$  diam com-

posito. Anamorphosis abest.

Holotypus: SUM 3134; colonia exsiccata in cultura ex solo sylvae, Chiba, in Japonia, 5.III.1996, a S. Uchiyama isolata et ea CBM conservata.

Etymology: Latin, *stellatus* = stellate, referring to the ascospore shape in the transverse section.

Colonies on PDA (1/2 strength) growing very restrictedly, attaining a diam of 20–22 mm in 35 d at 25°C, velvety, folded and wrinkled, consisting of a fairly tough basal felt, at first Greyish Green (M. 28D5), later becoming Dark Green (M. 28F3) or Olivaceous Black to Iron Grey (R) and granular from the development of abundant ascomata; margins rather narrow, entire; exudate small, clear; reverse Dark Green to Greenish Grey (M. 27F3–26F2) or Greenish Black (R). Colonies on OA growing slowly as on PDA but plane, thin, more or less floccose, producing abundant ascomata, overgrown by a loose network of white aerial hyphae, Dull Green (M. 26E3); reverse Brownish Grey to Greyish Orange (M. 11F2–6B5) or Brownish Vinaceous to Amber (R), with surrounding agar similarly colored. Colonies on PYE restricted, closely wrinkled, Brownish Orange to Brownish Grey (M. 6C4–6E2); ascomata not produced; reverse

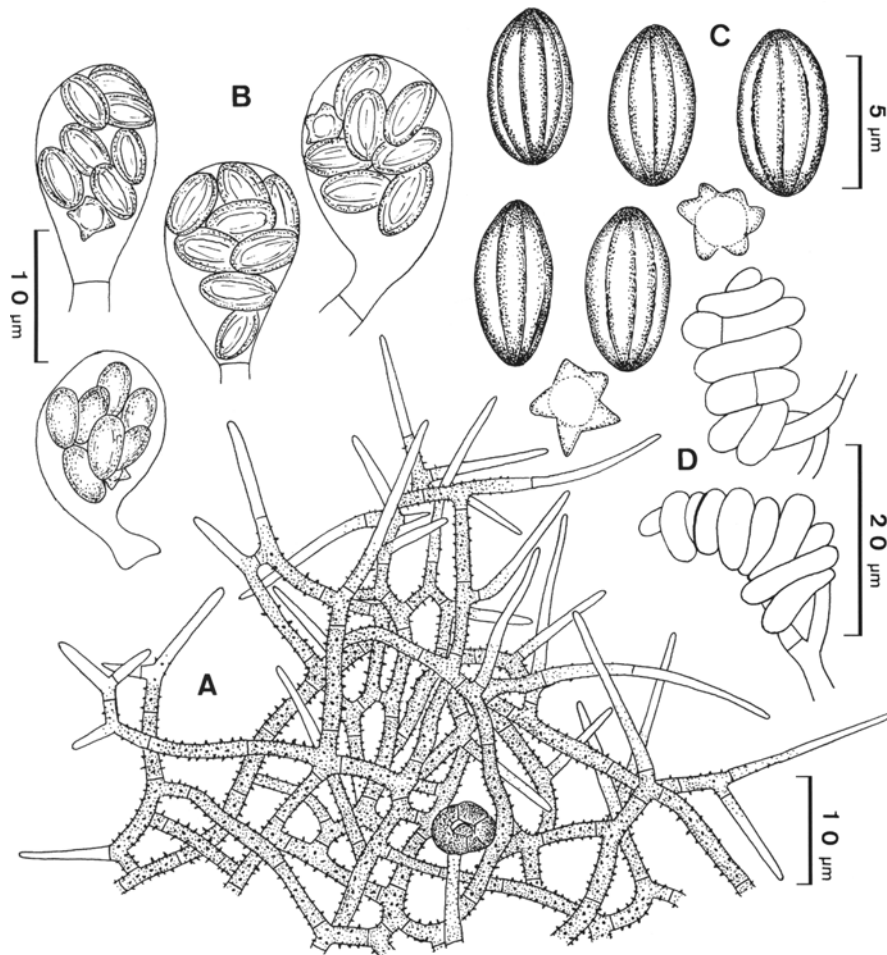


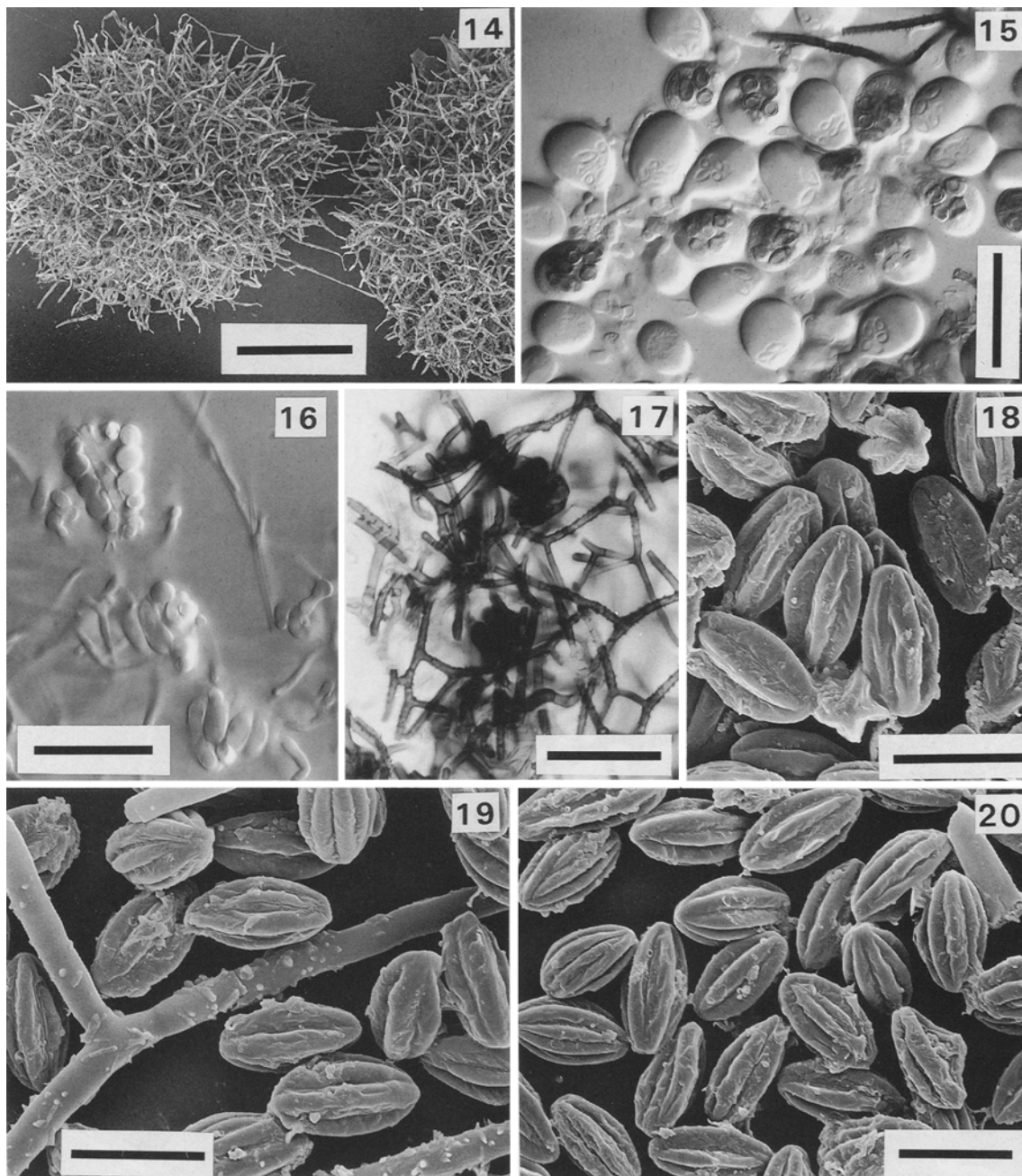
Fig. 13. *Myxotrichum stellatum* (SUM 3134).

A. Margin of ascoma showing appendages. B. Asci. C. Ascospores. D. Ascomatal initials.

Light Brown (M. 7D4).

Ascomata discrete or often confluent, more or less globose, 200–300(–500)  $\mu\text{m}$  in diam incl. appendages, brownish gray with a pale yellow centrum, maturing within 35–42 d; peridial hyphae hyaline to dark brown, thick-walled, septate, not inflating at the septum, 1.5–3  $\mu\text{m}$  in diam, smooth to asperulate, branched and anastomosed to form a loosely reticulate network, with internal branches with free ends terminating in a swollen or often coiled tip, peripherally ending in spine-like ap-

pendages; appendages pale brown, straight or somewhat bent, short, 12–40  $\times$  1.5  $\mu\text{m}$ , usually unbranched, septate, thick-walled, asperulate, terminating in a hyaline, pointed or bluntly rounded end. Asci hyaline, pointed or bluntly rounded end. Asci hyaline to yellowish brown, 8-spored, ovoid to broadly clavate or pyriform, 14–23  $\times$  8–12  $\mu\text{m}$ , short-stipitate, evanescent. Ascospores pale yellow to yellowish brown, fusiform to ellipsoidal, 4–6  $\times$  3–3.5  $\mu\text{m}$  (incl. ridges), striate in surface view, with 5–6 ridges running longitudinally, stellate or fluted in section.



Figs. 14–20. *Myxotrichum stellatum*.

14. Ascoma (SEM). 15. Asci. 16. Ascomatal initials. 17. Peridial hyphae with internal coiled branches. 18–20. Ascospores (SEM). Scale bars: 14 = 100  $\mu\text{m}$ ; 15–17 = 20  $\mu\text{m}$ ; 18–20 = 5  $\mu\text{m}$ .

Vegetative mycelium consisting of hyaline, branched, septate, smooth-walled, 1–3  $\mu\text{m}$  diam hyphae; ascomatal initials appear as swollen side branches arising from aerial hypha, soon tightly coiled and form a compact mass. Anamorph lacking.

Weakly cellulolytic.

At 37°C, growth is nil.

Holotype: SUM 3134, a culture isolated from forest soil in Mt. Nokogiriyama, Futtsu-shi, Chiba Pref., Japan, 5 March 1996, col. S. Uchiyama (CBM).

Note: *Myxotrichum stellatum* is easily distinguished from the other taxa by the ornamentation of its ascospores, which appears deeply striate in surface view because of the presence of five to six longitudinal ridges. Ascospore ornamentation of this type is similar to that of *Byssosascus striatosporus* (G. L. Barron et C. Booth) Arx (Barron and Booth, 1966; Arx, 1971; Currah, 1985). The ascomata of *B. striatosporus* are less highly specialized than the reticuloperidium made up of a mesh of thick-walled, dematiaceous peridial hyphae which characterize species of the genus *Myxotrichum* (Orr et al., 1963b; Currah, 1985). However, Sigler and Carmichael (1976) noted that ascospore production of *B. striatosporus* was consistently associated with the formation of dark brown, thick-walled, coalescing hyphae similar to the peridial hyphae of *Myxotrichum* ascomata, and regarded it as congeneric with the latter genus. Furthermore, Hambleton et al. (1998) have recently analyzed the phylogenetic relationships of 15 species in the hyphomycete genus *Oidiodendron* Robak and 10 species from the four genera in the Myxotrichaceae using the nucleotide sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA). The parasimony analysis showed that *Oidiodendron*, *Byssosascus* Arx, and *Myxotrichum* formed a monophyletic group within the Myxotrichaceae, and they therefore suggested that the generic concepts of *Myxotrichum* and *Byssosascus* need to be reexamined. Besides the ascomatal wall consisting of a distinctive reticuloperidium, this fungus can be distinguished from *B. striatosporus* by its larger asci (11–15  $\times$  5–7  $\mu\text{m}$  in *B. striatosporus*) and its broader ascospores (1.5–3  $\mu\text{m}$  in diam in *B. striatosporus*). In addition, *B. striatosporus* has an *Oidiodendron* anamorph.

*Gymnoascus uncinatus* Eidam, Cohn, Beitr. Biol. Pfl. 3: 292. 1880; Orr et al., Mycopathol. Mycol. Appl. 21: 5. 1963; Samson, Acta Bot. Neerl. 21: 524. 1972. Figs. 21–27

$\equiv$  *Myxotrichum uncinatum* (Eidam) J. Schroet., Cohn, Krypt.-Fl. Schles. 3(2): 212. 1893.

$\equiv$  *Uncinocarpus uncinatus* (Eidam) Currah, Mycotaxon 24: 186. 1985.

Colonies on YpSs agar growing slowly, attaining a diam of 36–38 mm in 28 d at 20°C, floccose, zonate, consisting of a thin basal felt with sparse white aerial hyphae, at first Pale Yellow (M. 3A3) or Primrose (R), gradually becoming Greyish Yellow to Greyish Orange (M. 4B6–5B6) or Apricot (R) due to the production of abundant ascomata borne in the loose mycelial felt, often

shading Greyish Green (M. 27C6) or Yellow Green (R) in age; margins thin, submerged; exudate large, brownish red; reverse Pastel Yellow to Brownish Orange (M. 3A4–6C4) or Ochreous (R). Colonies on keratin agar\* growing restrictedly, attaining a diam of 23–24 mm in 28 d at 20°C, thin, consisting of a submerged vegetative mycelium, producing abundant ascomata on the agar surface, lending to the colony an orange color as on YpSs; reverse uncolored. Colonies on PYE growing as on YpSs but conspicuously wrinkled and furrowed, floccose, consisting of a thick basal felt, Yellowish White (M. 3A2) or Straw (R); ascomata absent; reverse uncolored.

Ascomata discrete or often confluent, globose to subglobose, 120–400  $\mu\text{m}$  in diam excl. appendages, pale yellow to light orange, maturing within 35 d. Peridial hyphae thick-walled, smooth to asperulate, septate, 2–4(–5)  $\mu\text{m}$  in diam, not constricted or swollen at the septum, branched and anastomosed, forming a reticulate network (sometimes reduced to the undifferentiated peridial hyphae in the YpSs culture), with free apices forming short or elongate appendages; short appendages undifferentiated from peridial element, somewhat spine-like or boat-hook-shaped, blunt or subacute at the apex; elongate appendages yellow, usually simple but rarely branched near the base, smooth to asperulate, thick-walled with walls measuring about 1.5  $\mu\text{m}$  in thickness, septate only in lower part, uncinuate at the apex, (55–) 195–210  $\mu\text{m}$  long, 6–7  $\mu\text{m}$  wide at the upper part. Asci hyaline, 8-spored, globose to ovoid or pyriform, 7–10.5  $\times$  6.5–8  $\mu\text{m}$ , evanescent. Ascospores pale yellow, oblate, globose to subglobose in face view and ellipsoidal in side view, 3–3.5  $\times$  2–3  $\mu\text{m}$ , nearly smooth-walled.

Vegetative mycelium consisting of hyaline, branched, smooth-walled, septate, 1–4  $\mu\text{m}$  diam hyphae; ascomatal initials arising as morphologically similar lateral branches of the same hypha or separate hyphae, which soon coil several times. Anamorph lacking.

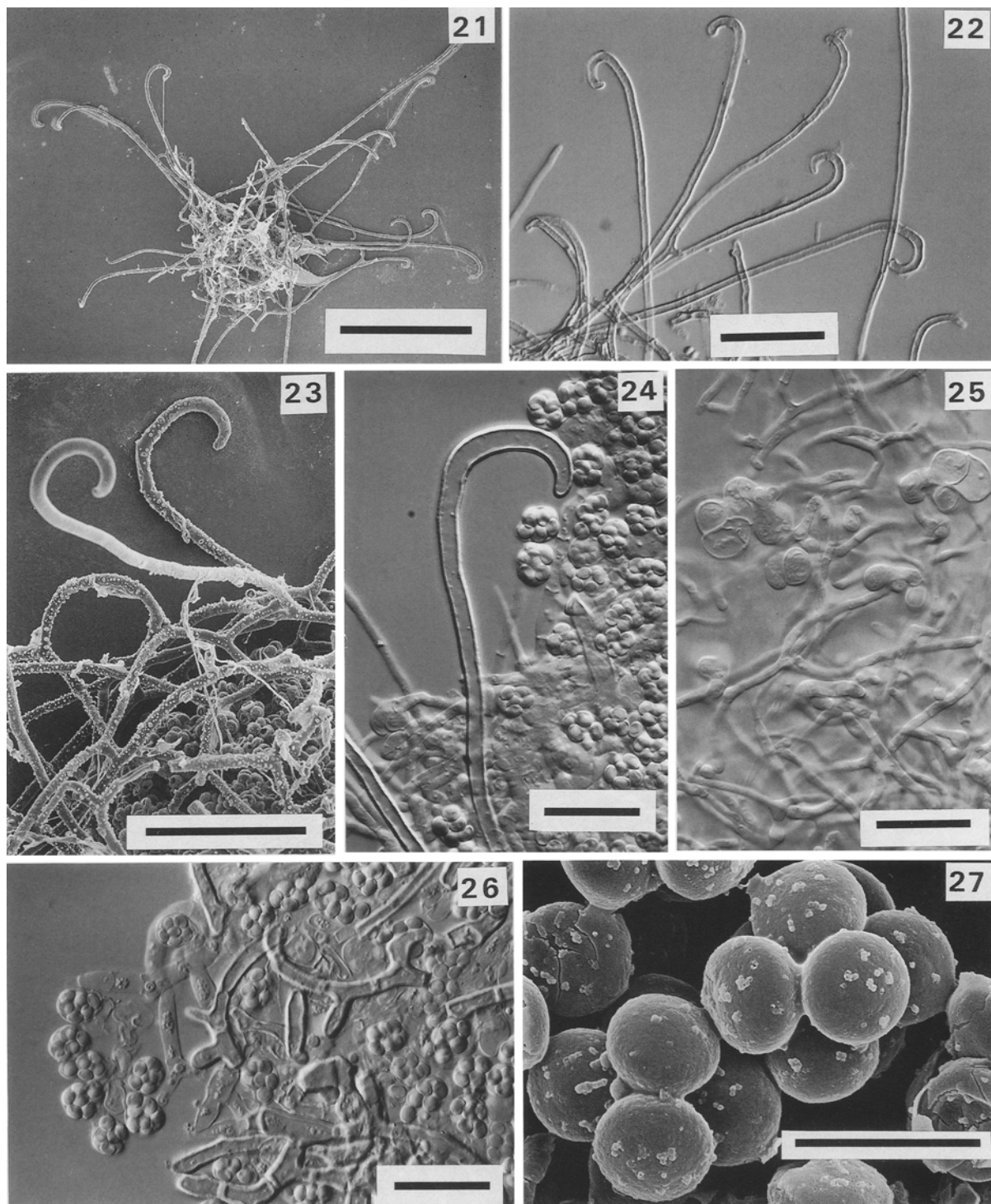
Keratinolytic.

At 25°C, colonies on YpSs become smaller; the only aerial mycelium is represented by tufts of developing ascomatal initials. At 37°C, growth is nil.

Specimen examined: SUM 3144, a culture isolated from forest soil, Upper Hot Springs, Banff National Park, Alberta, Canada, 29 September 1996, col. S. Uchiyama. The dried specimen has been deposited in the CBM.

Note: Sigler et al. (1998), in a discussion on the genus *Uncinocarpus*, stated that "*U. uncinatus*, which differs in forming a reticuloperidium, needs reevaluation, but the only available culture has not been reexamined in this study." The lattice-like peridium (reticuloperidium) of our isolate, as well as the coiled ascomatal initials and smooth-walled ascospores definitely demonstrate

\*  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1 g,  $\text{KH}_2\text{PO}_4$  1 g,  $\text{NH}_4\text{NO}_3$  2 g, trace element solution (below) 2.5 ml, distilled water 1,000 ml, keratin powder 3 g, agar 15 g (pH 6.5). Trace element solution:  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  2 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  0.04 g,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.04 g,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.04 g,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.08 g,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  0.08 g,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  0.24 g, distilled water 1,000 ml.



Figs. 21–27. *Gymnoascus uncinatus* (SUM 3144).

21. Ascoma (SEM). 22–24. Appendages (23. Appendages (SEM) and 24. Appendage and asci). 25. Ascosomal initials. 26. Asci and peridial hyphae. 27. Ascospores (SEM). Scale bars: 21=100  $\mu\text{m}$ ; 22, 23=50  $\mu\text{m}$ ; 24, 26=20  $\mu\text{m}$ ; 25=10  $\mu\text{m}$ ; 27=5  $\mu\text{m}$ .

that this species is representative of *Gymnoascus* (Orr et al., 1963a) rather than *Uncinocarpus* (cf. the note of *Arachniotus insolitus* in this paper). In addition, our isolate is not thermotolerant, with an optimum growth and

reproduction temperature of 20°C and no growth at 37°C. Thus our study showed that this fungus was excluded from *Uncinocarpus*.



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