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Taxonomic studies on new or critical fungi of non-pathogenic Onygenales 4

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Three noteworthy taxa of soil-borne onygenalean fungi are described and illustrated: *Kraurogymnocarpa trochleospora*, comb. nov., *Kuehniella aurea*, comb. nov., and *Nannizziopsis albicans*. In addition, *Aphanoascus boninensis*, which was previously described as a new species, is considered to be a synonym of *Uncinocarpus orissi*.

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

In a recent series of papers on the taxonomy of non-pathogenic Onygenales, we described a number of new taxa (Udagawa and Uchiyama, 1999a, 1999b, 2000b). This communication reports in part the results of the continued isolation of onygenalean fungi from soil samples collected in Japan and overseas. Three rarely encountered species in the Onygenales are described and illustrated herein: *Kraurogymnocarpa trochleospora* (Kuehn et Orr) Udagawa et Uchiyama, *Kuehniella aurea* (Eidam) Udagawa et Uchiyama, and *Nannizziopsis albicans* (Apinis) Guarro et al. We propose new combinations for the former two species, because of certain unusual or contradictory features that are considered worthy of special mention.

Color descriptions referred to with the letters M and R correspond to the color charts of Kornerup and Wanscher (1978) and Rayner (1970), respectively.

Taxonomy

Kraurogymnocarpa trochleospora (Kuehn et Orr) Udagawa et Uchiyama, comb. nov. Figs. 1–6

≡*Pseudoarachniotus trochleosporus* Kuehn et Orr, in Orr and Kuehn, Mycologia **64**: 58. 1972.

≡*Arachniotus trochleosporus* (Kuehn et Orr) Udagawa, Trans. Mycol. Soc. Japan **38**: 154. 1997.

Colonies on potato-carrot agar (PCA) growing rather slowly, attaining a diam of 24–29 mm in 21 d at 25°C, velvety to floccose, somewhat raised in central areas, consisting of a thin basal felt, producing ascomata in a dense layer, Reddish Orange to Reddish Brown (M. 7A7–9D6) or Apricot to Scarlet (R); margins thin, irregular; exudate clear, abundant; reverse uncolored to Greyish Orange or Reddish Orange (M. 5B4–7A7). Colonies on phytone yeast extract agar (PYE) growing more rapidly, attaining a diam of 30–34 mm in 21 d at 25°C, velvety to funiculose, often raised in central areas and radially sulcate, consisting of a compact basal felt, Greyish Green or Orange-Red (M. 30E5–8A7) or Grey Olivaceous to Scarlet (R); ascomata not produced; reverse Light Brown to Brown (M. 6D6–7E7) or Fawn (R), with surrounding agar similarly colored.

Ascomata superficial, discrete or often confluent, intermixed with aerial hyphae, yellowish red to brownish red, subglobose or irregular in shape, 25–200 μm in diam, maturing within 14–21 d. Peridial hyphae hyaline but incrusted with reddish granules, thin and smooth-walled, septate, often constricted at the septum, 1–3(–5.5) μm in diam, undifferentiated from the surrounding vegetative hyphae, branched and anastomosed, forming a telaperidium; appendages not produced. Asci usually 8-spored, singly borne, subglobose to ovoid or pyriform, 14–22 × 10–16 μm, hyaline to yellow, short-stipitate, somewhat persistent. Ascospores yellow to reddish yellow, lenticular, 5–7 × 4–6.5 μm incl. the crests, consisting of a central body 4–6 μm with two widely separate, ruffled equatorial crests about 1 μm wide and with convex surface showing a lobate-tuberculate ornamentation under the SEM.

Vegetative mycelium consisting of hyaline, branched, smooth-walled, septate, 1–4 μm diam hyphae; racquet hyphae present; anamorph lacking; ascomatal initials arising from two adjacent cells of the same aerial hypha or separating hyphae and becoming closely appressed to one another to give a clavate central initial and a coiling hypha.

At 37°C, growth on PCA and PYE is fairly slow; ascomata are not produced.

Cellulolytic.

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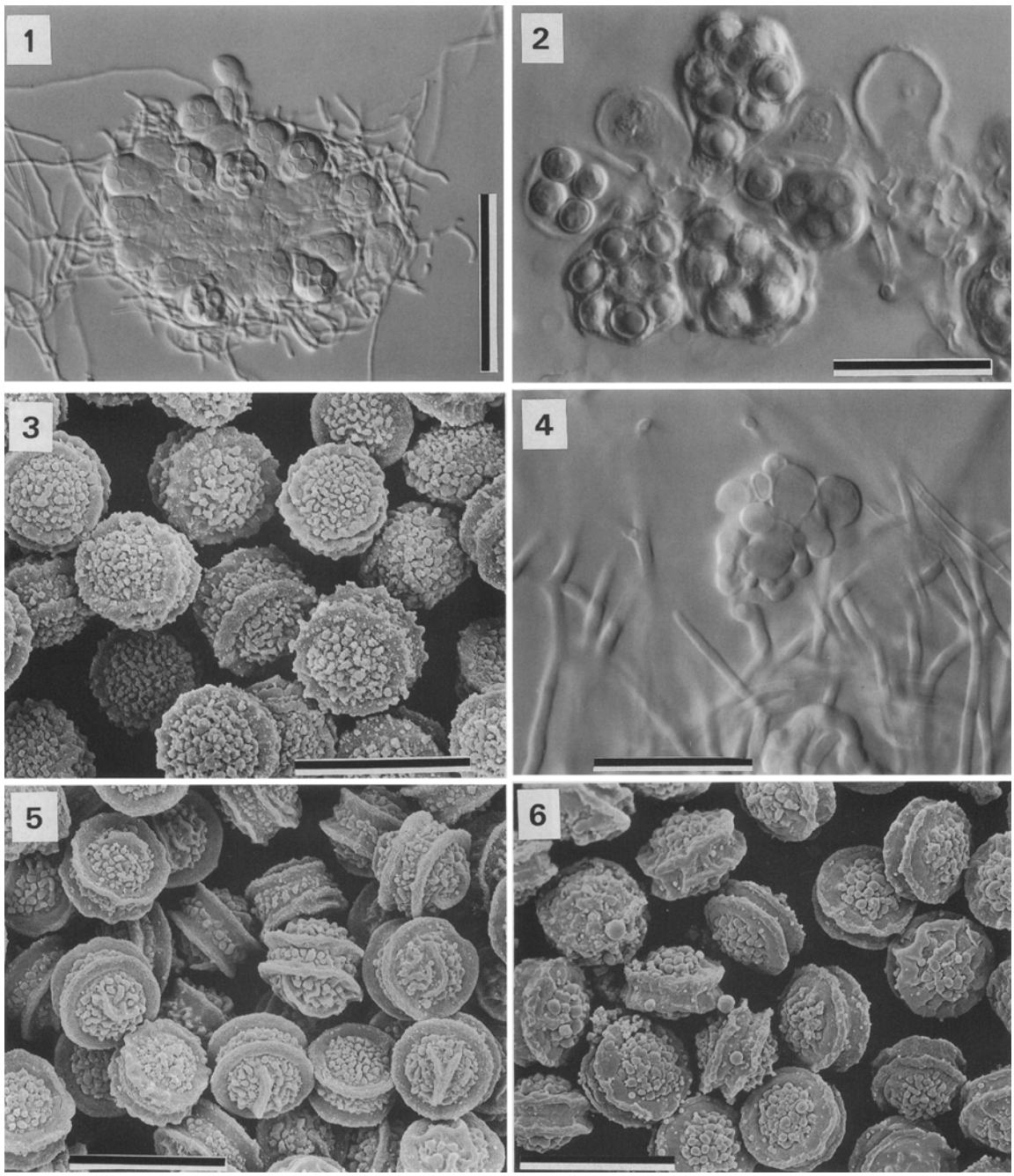
Distribution: USA, Indonesia, Monaco.

Specimens examined: SUM 3061, a culture isolated from garden soil, Bali island, Indonesia, 1 October 1994, col. S. Uchiyama (CBM), and SUM 3069, a culture isolated from garden soil, Monte Carlo, Monaco, 28 February 1995, col. S. Uchiyama (CBM).

Other specimen examined: NRRL A-16936, a culture derived from the holotype ex sandy-clay, Dugway, Utah, USA, col. G. F. Orr.

Note: Orr and Kuehn (1972) and Orr et al. (1977) originally reported spores of this fungus as follows: ascospores are yellow to yellow-brown en masse, yellowish viewed singly, spherical with an external rim in face view, 3.6–7.2 μm in diam, lenticular to pulley-shaped viewed on edge, 4.5–5.4 μm, grooved on the lateral axis, 2.7–3.6 μm wide, with crests on each side of the groove, 0.4–0.9 μm wide, and walls roughened. In his monographic study on the Onygenales, Currah (1985) regard-



Figs. 1–6. *Kraurogymnocarpa trochleospora*. 1. Ascoma (SUM 3069). 2. Asci (SUM 3069). 3. Ascospores (SEM, SUM 3069). 4. Ascomatal initials (SUM 3069). 5. Ascospores (SEM, SUM 3061). 6. Ascospores (SEM, NRRL A-16936). Scale bars: 1=50 μm; 2, 4=20 μm; 3, 5, 6=10 μm.

ed this fungus as synonymous with *Arachniotus ruber* (van Tieghem) Schroeter. Ascospores of the latter are yellow to orange, oblate, pulley-form with a shallow equatorial furrow and polar thickenings, smooth-walled and 4–6.6×2.8–4.4 μm (Kuehn and Orr, 1964; Apinis, 1964). Based on a scanning electron microscope (SEM) study on ascospores of this fungus, Udagawa (1997) showed the ascospores to have bivalvate bodies which are ornamented with two widely separated equatorial crests and lobate-tuberculate surfaces. Thus this fungus was defined as the second species of *Arachniotus* Schroeter.

However, further examination of this fungus during our current study and detailed comparison with *Kraurogymnocarpa lenticulospora* Udagawa et Uchiyama, which had been described as the type species of new genus in 1999 (Udagawa and Uchiyama, 1999a), led us to re-evaluate its assignment in the Onygenales. *Kraurogymnocarpa lenticulospora* resembles this fungus in showing rather slow growth on PCA; in developing strongly pigmented ascomata; and, particularly, in producing yellowish bivalvate ascospores with two widely separated equatorial crests and roughened walls. The genus *Kraurogymnocarpa* Udagawa et Uchiyama differs in the ascomata with a mesh-like peridium (reticuloperidium). The reticuloperidium has been emphasized as a feature that typifies "the old idea of the Gymnoascaceae." In his overview on peridial morphology and evolution in the prototunicate ascomycetes (Onygenales and Eurotiales), however, Currah (1994) stated that passive release of hyaline spores has led to a simplification and modification of peridial structure so that apparently unrelated groups share similar peridial types. Currah (1985, 1988), in re-sorting taxa traditionally distributed among the Gymnoascaceae, placed more emphasis on ascospore sculpturing characteristics and their degree of congruence with other characteristics, namely, anamorph morphology and enzymatic abilities.

This viewpoint is attracting increasing interest. In her taxonomic revision of the genus *Uncinocarpus* Sigler et Orr (Onygenales), Sigler et al. (1998) placed relatively little emphasis on the structure of the ascomatal peridium; the genus was emended to include keratinophilic fungi with discrete, globose gymnothecial ascomata without differentiated ascomatal hyphae and bearing uncinate, helical, or no appendages. In our most recent paper, we indicated a similar relationship with *A. ruber* and *A. insolitus* Udagawa et Uchiyama; the latter mainly differs in forming a reticuloperidium (Udagawa and Uchiyama, 2000b). Based on the ascospore morphology, this fungus is now considered to be more closely related to *K. lenticulospora* than to *A. ruber*, and a new combination is proposed. Molecular studies of these taxa may supply more evidence on their relationships.

Kuehniella aurea (Eidam) Udagawa et Uchiyama, comb. nov. Figs. 7–12

≡*Gymnoascus aureus* Eidam, Ber. Bot. Sect. Schles. Ges. **64**: 161. 1887.

≡*Arachniotus aureus* (Eidam) Schroeter, in Cohn, Krypt.-Fl. Schles. **3**(2): 210. 1893; Kuehn et al., Mycologia **56**: 863. 1964.

≡*Amauroascus aureus* (Eidam) von Arx, Persoonia **6**: 375. 1971.

Colonies on PCA growing restrictedly, attaining a diam of 35–37 mm in 27 d at 25°C, thin, consisting of a submerged vegetative mycelium, producing scattered ascomata and enveloping floccose hyphae on the agar surface in central colony areas, Pastel Yellow (M. 2A4) or Sulphur Yellow (R); conidiogenesis limited, hardly affecting the colony appearance; margins broadly submerged, thin; exudate produced as large droplets, clear; reverse uncolored. Colonies on diluted Sabouraud glucose agar (Takashio, 1972) growing somewhat slowly, floccose, more or less zonate; ascomata abundantly produced throughout the colony, Light Yellow (M. 2A5); conidiogenesis inconspicuous; reverse Light Yellow (M. 3A4). Colonies on PYE growing somewhat more rapidly, velvety, consisting of a thin basal felt characterized by a closely interlacing surface growth of trailing hyphae, Light Yellow (M. 2A5) or somewhat Sulphur Yellow (R); ascomata not produced; conidiogenesis inconspicuous; reverse Brown (M. 6D8) or Sienna (R).

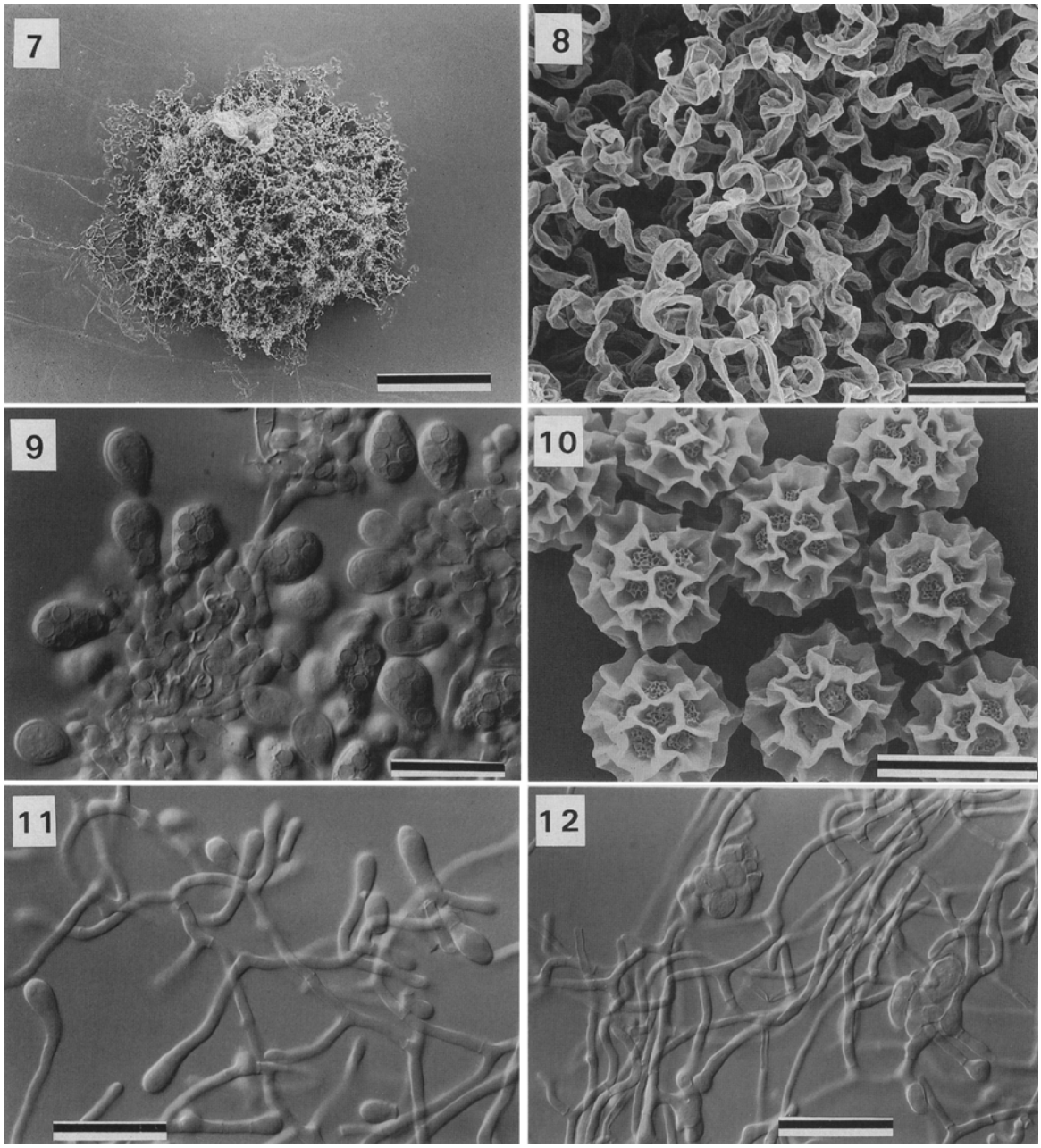
Ascomata discrete or often confluent, globose to subglobose, 400–700 μm in diam incl. the appendages, densely covered by aerial hyphae and conidia, yellow, with a grayish orange centrum, maturing within 21 d. Peridial hyphae at first scarcely differentiated from surrounding vegetative mycelium, hyaline, delicate, thin and smooth-walled, septate, 2–4 μm in diam, often swollen between the septa, branched and anastomosed, forming a loose network (telaperidium) in age. Appendages emerging from the periphery of matured ascoma, delicate, coiling spirally with 20 or more turns, often contorted, 1.5–2.5 μm in diam, septate, smooth-walled or incrusted with yellow granules; spirals 60–120 μm long, about 10 μm wide. Asci 8-spored, singly borne on a short stipe, pyriform to ellipsoidal, (12–)14.5–17.5×9.5–10.5(–12) μm, evanescent. Ascospores hyaline to pale yellow, globose to subglobose, 4–5 μm in diam, narrow-ridged alveolate-reticulate, with small punctae in the pits.

Vegetative mycelium consisting of hyaline, branched, septate, thin and smooth-walled, 1–2.5 μm diam hyphae, often intermixed with appendage-like structures; racquet hyphae present; ascomatal initials develop as swollen thick-walled branches from aerial hyphae.

Anamorph: *Chrysosporium*-like. Conidiophores micronematous. Aleurioconidia terminal or lateral, sessile or on short pedicels, one-celled, hyaline, clavate to pyriform, sometimes subglobose or obovoid, 6.5–17×4–8.5 μm, truncated at the base, apically rounded, thick-walled, smooth or verruculose, with broad or narrow basal scars. Arthroconidia hyaline, cylindrical or irregular shaped, often swollen to barrel-shaped, 6–21×1.5–4(–8) μm, truncated at both ends, aseptate or rarely 1-septate, smooth-walled or sometimes verruculose. Chlamydospores present, often in chains, globose to elongate, thick-walled, smooth, 6.5–9 μm in diam.

At 37°C, growth is nil.

Weakly keratinolytic.



Figs. 7–12. *Kuehniella aurea* (SUM 3149). 7. Ascoma (SEM). 8. Appendages (SEM). 9. Asci. 10. Ascospores (SEM). 11. Conidiogenous cells and conidia. 12. Ascomatal initials. Scale bars: 7=200 μm; 8, 9, 11, 12=20 μm; 10=5 μm.

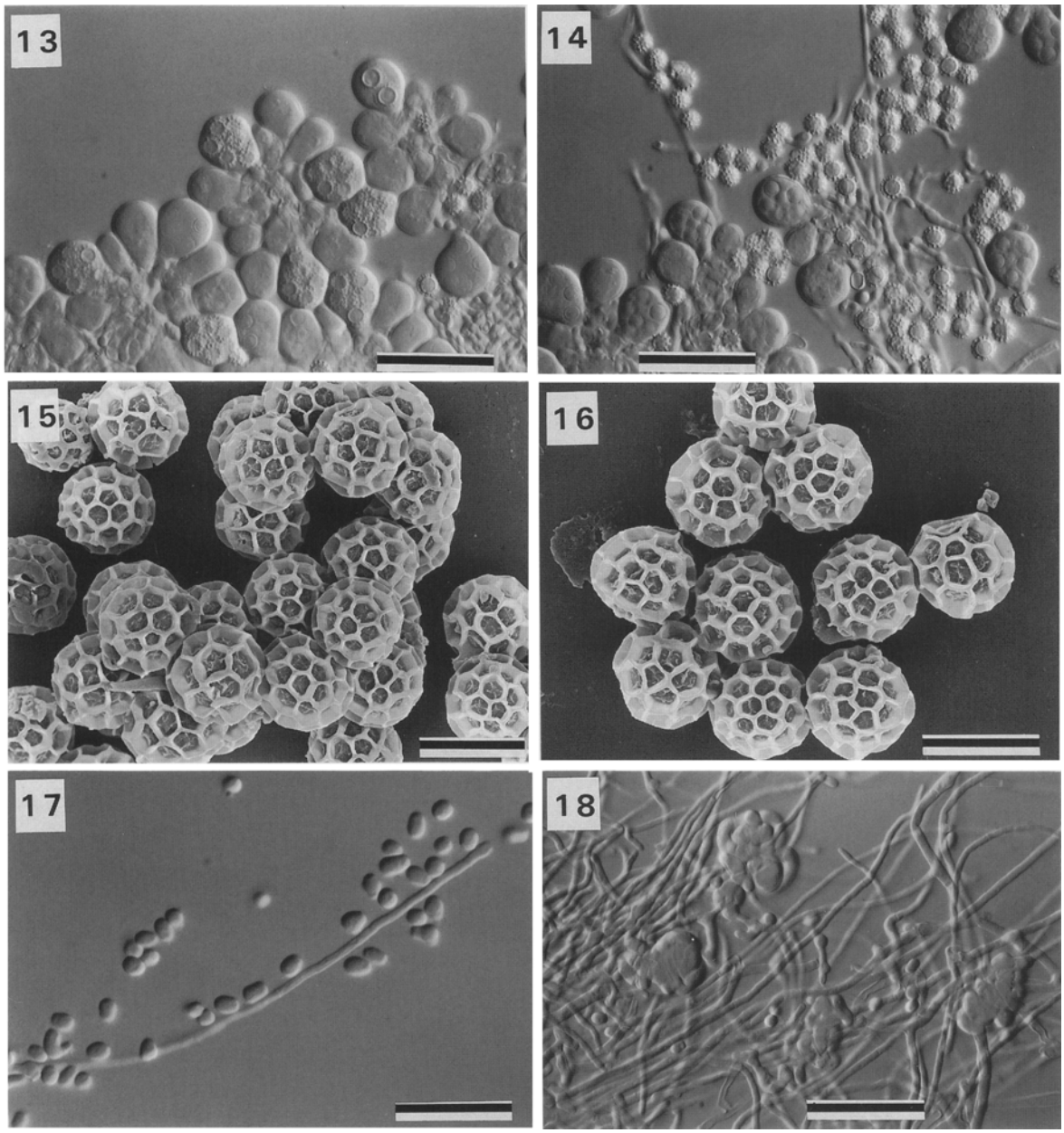
Distribution: Germany, Japan, USA, Egypt, Spain.

Specimen examined: SUM 3149, a culture isolated from forest soil, Takasuzuyama, Hitachi-shi, Ibaraki Pref., Japan, 2 May 1998, col. S. Uchiyama (CBM).

Other specimen examined: IFO 31763, a culture isolated from decayed wood in a cavity of a stump of *Cryptomeria japonica*, Asahi-mura, Higashitagawa-gun, Yamagata Pref., Japan, col. K. Tubaki, Aug. 1959.

Note: The monotypic genus *Kuehniella* was described by Orr (1976) for a fungus which was similar to *Amauroascus* Schroeter but which has white ascomata with undifferentiated peridial hyphae (telaperidium) and bearing helical appendages at the periphery. Orr described ascospores of *Kuehniella racovitzae* (Lagarde) Orr as being hyaline, globose, 2.3–4.2 μm diam, with thick, smooth walls. Subsequently Orr stated that *A. aureus* produces gymnothecia with sinuous to spiral filaments, but the ascospores are yellowish, ridged, possess a scalloped band and appear reticulate. Thus separation of *K. racovitzae* from *A. aureus* is plausible by light microscopy based on ascospore morphology.

Under SEM, however, ascospores of *K. racovitzae* were clearly punctate-reticulate (Currah, 1985; Udagawa and Uchiyama, 2000a). In addition, the ascospores



Figs. 13–18. *Nannizziopsis albicans* (SUM 3141). 13. Asci. 14. Asci and ascospores. 15, 16. Ascospores (SEM). 17. Conidia. 18. Ascomatal initials. Scale bars: 13, 14, 17, 18 = 20 μm; 15, 16 = 5 μm.

of *K. racovitzae* are often pale yellow, and placement of *A. aureus* in the genus *Kuehniella* is therefore not problematic.

In its formation of ascomata with spiral appendages and yellow, globose ascospores, *Apinisia graminicola* La Touche resembles this fungus (La Touche, 1968). However, the peridial elements of *A. graminicola* are distinctly present (vs. absent in *Kuehniella*) and at maturity reduced to a mass of disunited, thick-walled cells.

Nannizziopsis albicans (Apinis) Guarro, Cano et De Vroey, Mycotaxon **42**: 195. 1991. Figs. 13–18

≡*Arachniotus albicans* Apinis, Mycol. Pap. **96**: 45. 1964.

≡*Amauroascus albicans* (Apinis) von Arx, Persoonia **6**: 376. 1971.

≡*Arachniotus albicans* (Apinis) von Arx, The genera of fungi sporulating in pure culture, 2nd ed., p. 98. 1974.

Colonies on PCA growing rather rapidly, attaining a diam of 20–24 mm in 14 d at 25°C, floccose, plane, thin, with vegetative mycelium submerged and loose aerial hyphae, granular in surface appearance due to the production of abundant ascomata, white to Yellowish White (M. 4A2); margins irregular, broadly thin; conidiogenesis abundant, intermixed with ascomata; exudate few, clear; reverse Pale Yellow (M. 4A3) or Buff (R).

Colonies on oatmeal agar (OA) growing somewhat more rapidly than on PCA, 25–28 mm in 14 d at 25°C, but otherwise conforming to the above description. Colonies on PYE as on OA in rate of growth, radially sulcate, consisting of a thin mycelial felt, white; ascomata not produced; conidiogenesis abundant; reverse Greyish Orange (M. 5B4) or Ochreous (R).

Ascomata discrete or often confluent, globose to subglobose, 50–360 μm in diam, white to pale straw, maturing within 14–21 d. Peridial hyphae hyaline, thin and smooth-walled, septate, 1–3 μm in diam, undifferentiated from the surrounding vegetative hyphae, branched and anastomosed, forming a telaperidium; appendages not produced. Asci 8-spored, globose to subglobose, 10.5–12×9–10.5 μm, evanescent. Ascospores pale yellow, globose to subglobose, 4–5 μm in diam, thick-walled, broadly and regularly reticulate.

Vegetative mycelium consisting of hyaline, branched, septate, thin and smooth-walled, 1–2.5 μm diam hyphae; racquet hyphae present; ascomatal initials develop from two adjacent cells of the same parent hypha and become closely appressed to one another to give a club-shaped appearance.

Anamorph: *Chrysosporium*-like. Conidiophores micronematous. Aleurioconidia terminal, hyaline, subglobose to ovoid or pyriform, 2–5×1.5–4.8 μm, truncated at the base, apically rounded, smooth-walled. Arthroconidia hyaline, cylindrical, 4–12.5×1.5–2.5 μm, truncated at both ends, smooth-walled.

At 37°C, growth is nil.

Weakly keratinolytic.

Distribution: Germany, UK, Netherlands, Spain, USA, Paraguay.

Specimen examined: SUM 3141, a culture isolated from garden soil, Asunción, Paraguay, 14 July 1996, col. S. Uchiyama (CBM).

Note: The genus *Nannizziopsis* was introduced by Currah (1985) for the single species *Rollandina vriesii* Apinis (Apinis, 1970) which was characterized by white to grayish yellow ascomata, hyaline, branched and anastomosed, asperulate peridial hyphae with constrictions at septa and with appendages, hyaline, globose, punctate-recticulate ascospores, and a *Chrysosporium* anamorph. Thereafter, two species were added to the genus by Guarro et al. (1991): *Nannizziopsis hispanica* Cane et Guarro and *N. albicans*. *Nannizziopsis albicans* is superficially similar to the members of *Amauroascus*, because the asci are conglomerated within a telaperidium composed of loosely interwoven, undifferentiated hyphae and the ascospores are globose, punctate-reticulate. However, in comparison of the LSU rDNA sequences obtained from the representative strains of about 30 onygenalean species, Sugiyama and Mikawa (pers. commun.) concluded that *N. albicans* showed a closer relationship with *Apinisia* La Touche and *Shanorella* R. K. Benjamin than with genera of the Amauroascaceae such as *Amauroascus* and *Auxarthron* Orr et Kuehn.

Uncinocarpus orissi (B. Sur et G. R. Ghosh) Sigler et Fils, in Sigler et al., Can. J. Bot. **76**: 1624–1636. 1998.

≡*Pseudoarachniotus orissi* B. Sur et G. R. Ghosh, in Ghosh and Sur, Kavaka **12**: 67. 1985.

=*Gymnoascus arxii* Cane et Guarro, Stud. Mycol. **31**: 61. 1989.

=*Aphanoascus boninensis* Udagawa et Uchiyama, Mycoscience **40**: 283. 1999a.

Note: After the publication of our first report in the series of taxonomic studies on new or critical fungi of non-pathogenic Onygenales (Udagawa and Uchiyama, 1999a), we became aware of the description of *U. orissi* by Sigler et al. (1998, published in 1999). Our new fungus, *A. boninensis*, is particularly similar to *U. orissi*. The difference between the two taxa is the presence or absence of membranaceous peridial elements of the ascomata. The formation of membranaceous peridium in *A. boninensis* is not stable and often tends to be reduced or to disappear completely.

On the other hand, Cano and Guarro (1989) described the peridial structure of ascomata of *G. arxii*, a synonym of *U. orissi*, as follows: "peridium first made up of a tenuous mesh of hyphae 4–4.5 μm wide, eventually becoming flattened and turning into a membranaceous peridium which is hyaline to pale beige and composed of a layer of flattened cells."

Thus there seems to be no reason to maintain the separation of these taxa.

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