Short Communication

Isolation of *Roumegueriella rufula* as a new record from East Asia

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Roumegueriella rufula (Ascomycota; Hypocreales) isolated from a soil sample collected in Taiwan is described as a new record from East Asia. Some observations are recorded, particularly on cultural characteristics of a *Gliocladium* anamorph of this fungus, which has scarcely been reported since Bainier described both ascosporic and conidial forms as species of *Gliocladium*.

Key Words—ascomycete; Hypocreales; Roumegueriella rufula; soil fungus; Taiwan.

During a continuing survey of soil-borne microfungi for possible utilization in the pharmaceutical industry, a cleistothecial ascomycete with a Gliocladium anamorph was encountered on the agar plates of a soil sample collected from Taiwan and purely isolated on oatmeal agar. This ascomycete, characterized by flesh-colored globose ascomata with a membranaceous peridium, broadly clavate and very evanescent asci, and one-celled, yellowish brown, globose ascospores with prominent spines, was easily identified as Roumegueriella rufula (Berk. et Broome) Malloch et Cain (Dennis, 1978; Dennis and Wakefield, 1946; Ellis and Ellis, 1988; Hughes, 1951; Malloch and Cain, 1972). The fungus is apparently unreported from East Asian countries, although a second species of the genus, R. pulchella Udagawa, Uchiyama et Kamiya, was recorded by Udagawa et al. (1994) from soil in Okinawa, Japan.

The following description is based on isolate PF 1184 from soil of Taichung County in Taiwan.

Taxonomy

- Roumegueriella rufula (Berk. et Broome) Malloch et Cain, Can. J. Bot. 50; 64. 1972. Figs. 1–7 Basionym. Chaetomium rufulum Berk. et Broome,
 - Ann. Mag. Nat. Hist., Ser. 4, 11: 348. 1873.
 - *≡Lilliputia rufula* (Berk. et Broome) Hughes, CMI Mycol. Pap. **42**: 2. 1951.
 - *= Eurotium insigne* Wint. in Rabenh., Fung. Eur. 1732. 1874.
 - *≡Lilliputia insignis* (Wint.) Dennis et Wakefield, Trans. Br. mycol. Soc. 29: 145. 1946.
 - *⇒Roumegueriella muricospora* Speg., Rev. Mycol. 2: 18. 1880.
 - = Cephalotheca francisci D. Sacc., Malpighia 12: 206.

1898.

- *⇒Lilliputia gaillardii* Boud. et Pat., Bull. Soc. Mycol. France 16: 144. 1900.
- *Mycogala macrospora* Jaap, Verh. Bot. Ver. Brandenb. **52**(1): 19. 1910.
- Anam. *Gliocladium prolificum* Bain., Bull. Soc. Mycol. France **26**: 385–389. 1910.
- ≡ Cephalotheca prolifica (Bain.) Sacc. et Trotter, Syll. Fung. 22: 30. 1913.

Colonies on oatmeal agar growing rapidly, attaining a diameter of almost 45 mm in 14 d at 25 °C, floccose, plane, consisting of a thin basal felt, at first white, becoming Light Brown (M. 7D4, after Kornerup and Wanscher, 1978) or Fawn (Rayner, 1970) due to the abundant development of ascomata; conidiogenesis heavy but often in localized sectors; margins rather irregular, thin, submerged; exudate scattered, clear; reverse Reddish Brown (M. 8D5) or Sienna (R).

Ascomata non-ostiolate, superficial to semi-immersed or immersed, scattered, at first white, becoming Greyish Orange or Light Brown (M. 6B3-M. 7D4), globose to subglobose, 400-750 μ m in diam, intermixed with conidial heads and loosely covered by aerial hyphae, maturing within 28-35 d; peridium 50-120 μ m thick, straw-colored, semitransparent, membranaceous, several layered; outer layer of "textura prismatica" to "textura globulosa," composed of rounded to elongate cells measuring 12–20 μ m in diam; inner layer of "textura angularis," composed of hyaline, thin-walled, angular cells measuring 7.5–17.5 \times 5–10 μ m. Asci forming irregular fascicles, generally 8-spored, ovoid to broadly clavate, 70–84 \times 38–46 μ m, rounded above, without an apical apparatus, very evanescent; paraphyses lacking. Ascospores pale yellowish brown, globose to subglobose, $(17.5-)20-25(-27.5) \mu m$ incl. spines, thick-walled (ca.



Figs. 1-7. Roumegueriella rufula, PF 1184.

1. Part of an ascoma. 2. Young ascus and ascospores. 3. Ascospores in ascus. 4. Ascospores (SEM). 5. Penicillate conidiophores. 6. Upper portions of penicillate conidiophores. 7. Ellipsoidal conidia. Scale bars: $1 = 100 \ \mu\text{m}$; $2 - 4 = 20 \ \mu\text{m}$; $5 = 50 \ \mu\text{m}$; $6, 7 = 20 \ \mu\text{m}$.

 $2\,\mu m$), spinulose, consisting of a central body (15-22.5 μ m in diam) with splitting spines up to 3 μ m long, each containing one large guttule.

Mycelium composed of hyaline, branched, anastomosed, septate, smooth-walled, often swollen, guttulate, 2.5–20 μ m diam hyphae. Conidiophores mononematous, arising from the basal mycelium or aerial hyphae, frequently originating from a swollen cell of the hypha, erect, penicillately branched at the apex; stipes hyaline, 1-4-septate, $120-300 \times 7.5-12.5 \,\mu m$, up to 16 μ m wide near the base, or 45–100 μ m long that arise from the aerial hyphae, thick-walled, with walls generally smooth but appearing slightly rough near the base; penicilli biverticillate to quarterverticillate, with cellular elements smooth and closely appressed to form a very compact fruiting head, sometimes composed of irregular branching systems. Branches 2-8 in the verticil, 20-40×6-12.5 μm. Metulae 6-10 in the verticil, 9-20 \times 2.5-6 μ m. Phialides cylindrical to ampulliform, 4-6 in the verticil, $10-20 \times 2.5-4(-6.5) \mu m$, with narrow collula. Conidia aggregated into globose, gloeoid heads, hyaline, at first cylindrical, then ellipsoidal, 5-10(-14) \times 2.5-4 μ m, sometimes ovoid to lemonshaped, $4-7.5 \times 3-6.5 \,\mu m$, with walls smooth.

Colonies on potato-carrot agar growing rapidly, attaining a diameter of 33–46 mm in 14 d at 25°C, floccose, plane, consisting of a thin layer of abundant conidial heads and fewer ascomata than on the oatmeal agar, Light Brown (M. 7D4); reverse Reddish Brown (M. 8D5).

At 37°C, growth is nil.

Known distribution: France, Germany, India, Mexico, Taiwan, UK, USA.

Specimen examined: PF 1184, in a culture isolated from cultivated soil, Houli, Taichung County, Taiwan, 20 September 1993, collected by T. Yaguchi.

Specimens examined (for comparison): TRTC (H. Rehm - Ascomyceten 1200), on horse dung in compost, Angustenburg near Nossen, Sachsen, Germany, May 1887, Krieger, as *Eurotium insigne*; TRTC, on decaying seaweeds, Kittery Point, Maine, USA, 3 July 1918, R. Thaxter, as *Lilliputia*; TRTC (RFC 6468), on partridge dung, Tamsel, Brandenburg, Germany, 14 February 1935, P. Vogel, as *Penicillium insigne*; TRTC (CBS 276.59), culture from axis deer dung, California, USA, 1958, S. Stribling, as *Lilliputia rufula* with a *Gliocladium* anamorph; and TRTC 37774, on burro dung, Cd. del Maiz, San Luis Potosi, Mexico, 19 August 1960, R.F. Cain, as *Lilliputia rufula*.

The shape of asci of this fungus is very broadly clavate, the evanescent ascus wall is typically of the cleistothecial ascomycetes, and the ascospores are soon liberated by the disappearance of the walls. Thus the ascomatal cavity is eventually filled with free ascospores. The ascospore number in the ascus is usually eight, but numbers of four and even two have been observed in cultures.

Roumegueriella rufula has been found commonly on various kinds of animal dung as saprophytes: the dung of

axis deer, burrow, fowl, horse (often on mushroom compost), kangaroo, partridge and sheep (Massee and Salmon, 1901). A detailed historical account of R. rufula has been presented by Hughes (1951). Regarding the Gliocladium anamorph, Hughes stated only that "Bainier (1910) described his new species Gliocladium prolificum and found that it readily produced a considerable number of perithecia on various substances; he gave a description of the perithecia and remarked that the conidial apparatus is perhaps the same as that which Winter had recorded as G. penicillioides Corda in 1873." The resemblance between the Gliocladium anamorph of R. rufula and G. penicillioides, however, is entirely superficial; as was described by Samuels (1976) and Seifert (1985), G. penicillioides is the anamorph of Sphaerostilbella aureonitens (Tul.) Seifert, Samuels et W. Gams (=Hypomyces aureonitens Tul.) and the description given by Seifert indicates that G. penicillioides is different from this fungus in having much smaller conidial apparatus.

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Literature cited

- Bainier, G. 1910. Mycothèque de l'Ecole de Pharmacie. xxxii. Bull. Soc. Mycol. France **26**: 385–389.
- Dennis, R. W. G. 1978. British Ascomycetes. J. Cramer, Vaduz.
- Dennis, R. W. G. and Wakefield, E. M. 1946. New or interesting British fungi. Trans. Br. Mycol. Soc. 29: 141–166.
- Ellis, M. B. and Ellis, J. P. 1988. Microfungi on miscellaneous substrates. An identification handbook. Croom Helm, London.
- Hughes, S. J. 1951. Studies of Microfungi. VIII. Orbicula and Lilliputia. CMI Mycol. Pap. 42: 1–27.
- Kornerup, A. and Wanscher, J. H. 1978. Methuen handbook of colour, 3rd ed., Eyre Methuen, London.
- Malloch, D. and Cain, R. F. 1972. New species and combinations of cleistothecial Ascomycetes. Can. J. Bot. 50: 61–72.
- Massee, G. and Salmon, E.S. 1901. Researches on coprophilous fungi. I. Ann. Bot. 15: 313–357.
- Rayner, R. W. 1970. Mycological colour chart. Commonw. Mycol. Inst., Kew and British Mycological Society.
- Samuels, G. J. 1976. A revision of the fungi formerly classified as *Nectria* subgenus *Hyphonectria*. Mem. New York Bot. Gard. **26**(3): 1–126.
- Seifert, K. A. 1985. A monograph of *Stilbella* and some allied hyphomycetes. Stud. Mycol. **27**: 1–235.
- Udagawa, S., Uchiyama, S. and Kamiya, S. 1994. A new species of *Roumegueriella*. Mycoscience **35**: 409–412.