A new species of *Roumegueriella*

Shun-ichi Udagawa¹⁾, Shigeru Uchiyama²⁾ and Seigo Kamiya²⁾

- ¹⁾ Nodai Research Institute, Tokyo University of Agriculture, 1-1, Sakuragaoka 1-Chome, Setagaya-ku, Tokyo 156, Japan
- ²⁾ New Drug Discovery Research Laboratories, Tsukuba Research Institute, Banyu Phamaceutical Co., Ltd., 3, Ookubo, Tsukuba-shi, Ibaraki 300-33, Japan

Accepted for publication 30 October 1994

A new species of *Roumegueriella* (Ascomycetes; Hypocreales), *R. pulchella*, is described and illustrated. This fungus is characterized by its rapid growth on Czapek-yeast extract and YpSs agars at 37°C, bright yellow non-ostiolate ascomata, translucent membranaceous peridium, broadly clavate asci, and hyaline one-celled subglobose-ovoid ascospores ornamented with prominent spines. The holotype was isolated from soil in a sugarcane field in Okinawa, Japan.

Key Words-ascomycete; Hypocreales; Japan; Roumegueriella pulchella; soil fungus.

The genus Roumegueriella Speg. with the type species R. rufula (Berk. et Br.) Malloch et Cain (Malloch and Cain, 1972) is rather a rare fungus in the Hypocreales. It was treated in detail by Hughes (1951), who described it under the name Lilliputia rufula (Berk. et Br.) Hughes, having transferred it from Chaetomium rufulum Berk. et Br. Malloch placed Roumegueriella in the Hypocreaceae (Malloch, 1970; Benny and Kimbrough, 1980), whereas Eriksson and Hawksworth (1993) suggest it to be placed with a "?" in the Hypocreaceae in the 1993 Outline of the Ascomycetes. Based on analysis of the 28S rDNA gene sequence, Rehner and Samuels (1994) recently stated that "the teleomorph morphology of *R. rufula* provides little indication of its position within the Hypocreales, but the rDNA phylogeny clearly shows it to be derived from within "Group 2" (a clade grouped around Nectria ochroleuca (Schw.) Berk. (anam.=Gliocladium roseum Bain.)).

During the course of a continuing study of soil-borne ascomycetes from Japan, a bright yellow cleistothecial ascomycete was encountered and isolated on potato-dextrose agar. The fungus was characterized by the following: ascomata which are superficial, globose, non-ostiolate and yellowish; translucent membranaceous peridium; broadly clavate asci without an apical apparatus; hyaline, subglobose to ovoid ascospores with prominent spines. In accordance with this profile, our isolate is included in the genus *Roumegueriella*. An examination of the five materials of *R. rufula* which are deposited at the University of Toronto Cryptogamic Herbarium (TRTC) disclosed several significant differences between our isolate and *R. rufula*. We therefore propose a new species, *Roumegueriella pulchella*, to accommodate the fungus.

Roumegueriella pulchella Udagawa, Uchiyama et Kamiya, sp. nov. Figs. 1, 2 (A-F) Coloniae in agaro "Czapek-yeast extract (CYA)" effusae, floccosae, planae, ex mycelio basali coacto tenuiter constantes, granulares, ascomatibus abundantibus formantes, luteolae; reversum dilute aurantiacum vel croceum. Coloniae in agaro maltoso maxime restrictae. Coloniae in agaro "YpSs" modice crescentes, velutinae, planae, ex mycelio basali coacto tenuiter constantes, primo luteolae, deinde dilute brunneae vel avellaneae; reversum dilute aurantiacum vel croceum.

Ascomata non ostiolata, superficialia, dispersa, globosa vel subglobosa, 100-200 μ m diam, flava, cum hyphis hyalinis, tenuibus, rectis, flexuosis, simplicibus, septatis, levibus obtecta, lente maturescentia; peridium 5-7.5 μ m diam, hyalinum, translucens, membranaceum, ex "textura epidermoidea" et "textura angularis" compositum, circa tristratum. Asci octospori, late clavati, (16-)18-26 × 12-18 μ m, irregulariter fasciculati, sine apparatu apicali, brevi-stipitati, evanescentes; paraphyses indistinctae. Ascosporae hyalinae, subglobosae vel ovoideae, 6.5-8 × 5.5-7 μ m (cum spinis), manifeste spinosae.

Mycelium ex hyphis hyalinis vel dilute flavo-brunneis, ramosis, saepe anastomosantes, septatis, asperatis, 1-2.5(-4) μ m diam compositum; anamorphosis abest.

Ubiquinonum majus: Q-9.

Holotypus BF 46827, colonia exsiccata in cultura ex solo sativo in Insula Iheya, Iheya-mura, Shimajiri, Okinawa, in Japonica, 14.ix.1993, a S. Uchiyama et S. Kamiya isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis Chiba (CBM) conservata. Isotypus: TRTC.

Etymology: from Latin, *pulchella*=beautiful and little, referring the ascospore characters.

Colonies on CYA growing rapidly, attaining a diam of



Fig. 1. Roumegueriella pulchella (BF 46827). A. Ascoma. B. A part of ascomatal peridium. C. Asci. D. Ascospores. E. Ascomatal initials.

34-35 mm in 14 days at 25°C, floccose, plane, consisting of a thin mycelial felt, granular in appearance due to the development of abundant ascomata, with sparse covering of aerial hyphae, Light Yellow (M. 4A4, after Kornerup and Wanscher, 1978) or Pale Luteous (Rayner, 1970); margins largely submerged, entire; exudate abundant, clear; odor strongly pronounced, suggestive of butyric acid; reverse Light Orange (M. 5A4) or Saffron (R).

Colonies on malt extract agar growing very restrictedly, attaining a diam of 5-8 mm in 14 days at 25°C, with surface growth of white aerial hyphae and limited ascomata.

Colonies on YpSs agar growing moderately, attaining a diam of 28-30 mm in 14 days at 25°C, velvety, plane, consisting of a thin basal felt, producing abundant ascomata on the felt, Light Yellow (M. 4A4) or Pale Luteous (R), then becoming Light Brown (M. 6D5) or Hazel (R) in 28 days; margins broad, white; exudate and odor as described on CYA; reverse Light Orange (M. 6A4) or Saffron (R).

Ascomata non-ostiolate, superficial, scattered, globose to subglobose, 100-200 μ m in diam, at first hyaline, becoming yellow in age, sparsely covered with hyaline, delicate, straight or flexuous, septate, smoothwalled, 37.5-115×1-2 μ m hyphae, maturing within 28 days; peridium 5-7.5 μ m in diam, hyaline, translucent, membranaceous, of "textura epidermoidea" and "textura angularis," about 3-layered; outer layer consisting of irregular shaped, thick-walled, 2.5-5 μ m wide cells; middle and inner layers consisting of angular, thinwalled, 4-16×3-12 μ m cells. Asci 8-spored, thin, broadly clavate, (16-)18-26×12-18 μ m, forming irregular fascicles, rounded above, without an apical apparatus, narrowed to a short stipe (stipes up to 5 μ m long)



Fig. 2. A-F. *Roumegueriella pulchella* (BF 46827). A. Ascoma. B. A part of ascomatal peridium. C. Ascomatal initial. D. Asci. E. Asci and ascospores. F. Ascospores (SEM). G. *Roumegueriella rufula* (H. Rehm-Ascomyceten 1200). Ascospores (SEM). Scale bars: A=50 μm; B-E, G=20 μm; F=5 μm.

with crozier at the base, evanescent at maturity; paraphyses indistinct. Ascospores hyaline, subglobose to ovoid, $6.5-8 \times 5.5-7 \,\mu$ m including the spines, spinose, consisting of a central body $5-6.5 \times 4-5 \,\mu$ m with prominent spines up to $1.5 \,\mu$ m long.

Mycelium consisting of hyaline to pale yellowish brown, branched, often anastomosed, septate, rough-

ened with pigmented granules, $1-2.5(-4) \mu m$ diam hyphae; ascomatal initials consisting of swollen side branches of the vegetative hyphae, becoming surrounded by large elongated cells arising from the neighboring hyphae; anamorph not seen.

Major ubiquinone: Q-9.

At 37°C, 14 days, colonies on CYA and YpSs agar

are 60-64 mm in diam, plane, straw in color; ascomata abundantly produced; reverse in the same shades as the colonies at 25°C.

Specimen examined: BF 46827 (holotype), in dried culture isolated from soil in sugarcane field, Iheya Island, Iheya-mura, Shimajiri-gun, Okinawa-ken, Japan, 14 September 1993. The holotype has been deposited with the Natural History Museum and Institute, Chiba (CBM), Japan. Isotype: TRTC.

The morphology of the following specimens of *Roumegueriella rufula* has been compared with that of the holotype BF 46827: on horse dung in compost, Angustenburg near Nossen, Sachsen, Germany, May 1887, Krieger, H. Rehm-Ascomyceten 1200, as *Eurotium insigne* (TRTC); on decaying seaweeds, Kittery Point, Maine, U.S.A., 3 July 1918, R. Thaxter, as *Lilliputia* (TRTC); on partridge dung, Tamsel, Brandenburg, Germany, 14 February 1935, P. Vogel, RFC 6484, as *Penicillium insigne* (TRTC); culture from Axis deer dung, California, U.S.A., 1958, S. Stribling, CBS 276.59 (=IMI 073.837) with anam. *Gliocladium*, as *Lilliputia rufula* (TRTC); and on burro dung, Cd. del Maiz, San Luis Potosi, Mexico, 19 August 1960, R.F. Cain, as *Lilliputia rufula*, TRTC 37774.

Roumegueriella pulchella is the second species known in this genus. Its most characteristic features are yellow, non-ostiolate ascomata with translucent membranaceous peridium, broadly clavate asci without an apical appratus, and one-celled, subglobose to ovoid, spinose ascospores.

Although the ascospores of most specimens of *R. ruf-ula* are approximately 16-24 μ m in diam (Fig. 2-G), variability of ascospore dimensions in the previous collections has been observed (Dennis and Wakefield, 1946; Hughes, 1951). The difference in the thickness of the ascomatal peridium is also remarkable: in the peridium of the specimen CBS 276.59 is 100 μ m while those of the

specimens H. Rehm-Ascomyceten 1200 and R. Thaxter are less than 20 μm thick.

In spite of this morphological variability in *R. rufula*, *R. pulchella* is readily distinguished by its small, subglobose to ovoid ascospores. Since *R. rufula* is sometimes accompanied by a *Gliocladium* anamorph, the cultures of *R. pulchella* were incubated on various media and under different conditions in an attempt to induce its anamorphic state. Despite our efforts no anamorph could be observed.

Acknowledgements — We are very much obliged to Dr. J. C. Krug of the University of Toronto for the loan of herbarium materials.

Literature cited

- Benny, G. L. and Kimbrough, J. W. 1980. A synopsis of the order and families of Plectomycetes with keys to genera. Mycotaxon 12: 1-91.
- Dennis, R. W. G. and Wakefield, E. M. 1946. New or interesting British fungi. Trans. Br. Mycol. Soc. 29: 141–166.
- Eriksson, O. E. and Hawksworth, D. L. 1993. Outline of the Ascomycetes-1993. Systema Ascomycetum 12: 51-257.
- Hughes, S. J. 1951. "Studies of microfungi. VIII. Orbicula and Lilliputia," Mycol. Pap. No. 42, Commonw. Mycol. Inst. Kew. 28p.
- Kornerup, A. and Wanscher, J. H. 1978. "Methuen handbook of colour," 3rd ed., Eyre Methuen, London. 252p.
- Malloch, D. 1970. "The genera of cleistothecial Ascomycota," Ph.D. Diss., University of Toronto, Toronto. 672p.
- Malloch, D. and Cain, R. F. 1972. New species and combinations of cleistothecial Ascomycetes. Can. J. Bot. 50: 61– 72.
- Rayner, R. W. 1970. "Mycological colour chart," Commonw. Mycol. Inst., Kew and British Mycological Society.
- Rehner, S. T. and Samuels, G. J. 1994. Taxonomy and phylogeny of *Gliocladium* analyzed from nuclear large subunit ribosomal DNA sequences. Mycol. Res. 98: 625–634.