New and interesting species of *Emericella* from Brazilian soil

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Among the ascomycete isolates from soil and armadillo dung collected in São Paulo State, Brazil, a new species of *Emericella*, *E. montenegroi* and a new variety of *E. rugulosa*, *E. rugulosa* var. *lazulina* are described and illustrated. *Emericella montenegroi* differs from the other known species of the genus in having ascospores with an incompletely reticulate or ribbed ornamentation on the convex walls. *Emericella rugulosa* var. *lazulina* differs from the type variety in having blue to violet color of ascospores. Isolations of *E. corrugata* and *E. foveolata* are also reported as a South American record. A synoptic key to all accepted species of the genus is provided.

Key Words——Aspergillus montenegroi; Brazil; Emereicella corrugata; Emericella foveolata; Emericella montenegroi; Emericella rugulosa var. lazulina; soil fungi.

During a survey of pathogenic and mycotoxin-producing fungi in the South America (Brazil, Colombia and Venezuela) conducted from 1986, uncommon isolates of *Emericella* were collected from Brazilian soil and armadillo dung.

From the collection, isolate 93-BS-1012-4 is characterized by production of ascospores with an incomplete reticulate ornamentation on the convex walls. The isolate proved to be sufficiently different from all described species of Emericella (Christensen et al., 1978; Christensen and States, 1982; Horie, 1978, 1979, 1980; Horie and Udagawa, 1995; Horie et al., 1989, 1990; Kong and Qi, 1986; Malloch and Cain, 1972; Mehrotra and Prasad, 1969; Raper and Fennell, 1965; Samson and Mouchacca, 1974, 1975; Udagawa and Horie, 1976; Udagawa and Muroi, 1979) to warrant its description as a new species. Another Emericella isolate, 93-BS-42, was identified as E. rugulosa (Thom & Raper) C. R. Benjamin, although it differs distinctly from the typical strains in its ascospore color. The grayish magenta to grayish violet color serves to distinguish it from the type variety.

Cultures of the new species and the new variety as well as dried materials are deposited at the Natural History Museum and Institute, Chiba (CBM). Two known taxa of *Emericella* are also described in this paper.

Emericella montenegroi Horie, Miyaji & Nishimura, sp. nov.
Figs. 1-5

Coloniae in agaro Czapekii celeriter crescentes, aurantiaco-albae vel flavo-griseae; cleistothecia abundanter producentia, granularia; reversum subaurantiacum vel brunneo-aurantiacum.

Coloniae in agaro maltoso effusae, subflavae vel

griseo-virides; cleistothecia abundanter producentia, granularia, sed capitula conidica sparsa; reversum subflavum vel griseo-flavum.

Cleistothecia superficialia, rubro-purpurea vel valde rubro-brunnea, globosa vel subglobosa, 200-450 μ m diam, cum cellulis dictis "hülle" numerosis, crassitunicatis, globosis vel subglobosis, 13-20 μ m diam circumcincta; peridium griseo-flavum vel obscure rubrum, e stratis duobus vel tribus compositum. Asci octospori, subglobosi vel ovoidei, $8.5-12\times7-8~\mu$ m diam, evanescentes. Ascosporae primo hyalinae vel dilute flavobrunneae, deinde obscure rubrae vel rubro-brunneae vel griseo-rubrae, lenticulares, $3.6-4.8\times3.2-3.6~\mu$ m, cristis aequatorialibus duabus plicatis praeditae, parte convexa imperfecte reticulata vel costata ornatae. Status anamorphus: *Aspergillus montenegroi*.

Holotypus CBM-FA-0669, colonia exsiccata in cultura ex solo, Botucatú, São Paulo, 21. VII. 1993, a Y. Horie isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis, Chiba (CBM) conservata.

Etymology: named as a memorial to Honorary Professor Mario R. Montenegro, Laboratory, Biosciences Institute "Faculde de Medicina, Universidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil, eminent medical mycologist.

Anamorphosis: Aspergillus montenegroi Horie, Miyaji & Nishimura, anam. sp. nov.

Capitula conidica griseo-olivaceo-viridia vel obscure viridia, brevi-columnaria, $65-110\,\mu\mathrm{m}$ longa, 35-55 (-80) $\mu\mathrm{m}$ crassa. Conidiophora brunneo-aurantiaca vel griseo-brunnea, plus minusve sinuata, levia, incrassata, usque $180\,\mu\mathrm{m}$ longa, $4-5\,\mu\mathrm{m}$ diam. Vesiculae brunneo-aurantiacae vel griseo-flavae, hemisphaericae vel

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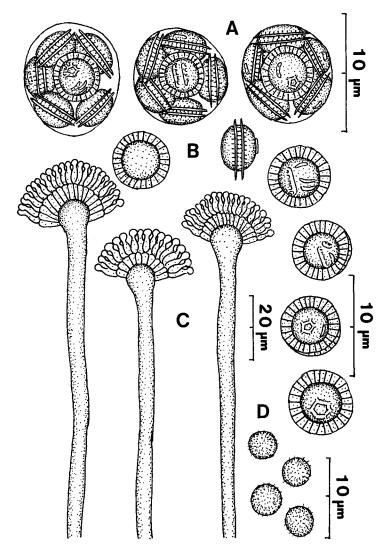


Fig. 1. Emericella montenegroi.
A. Asci. B. Ascospores. C. Aspergilla. D. Conidia.

lageniformes, 8-11 μ m diam. Aspergilla biseriata; metulae 5-6×2-2.5 μ m; phialides rubro-albae vel rubrogriseae, 6-7×2-2.5 μ m. Conidia griseo-viridia, globosa vel subglobosa, minute asperata vel echinulata, 3-4 μ m diam. Status teleomorphus: *Emericella montenegroi*.

Holotypus CBM-FA-0669, loc. cit.

Colonies on Czapek agar spreading broadly, attaining a diameter of 65–69 mm in 14 d at 25 °C, Orange White (5A2, after Kornerup and Wanscher, 1978) to Yellowish Grey (4B2), consisting of a fairly tough mycelial felt, deeply furrowed, velvety, producing abundant cleistothecia in a dense layer on the mycelial felt; conidial heads few in number; reverse Pale Orange (5A3) to Brownish Orange (6C6).

Colonies on Czapek-yeast extract agar (CYA) spread-

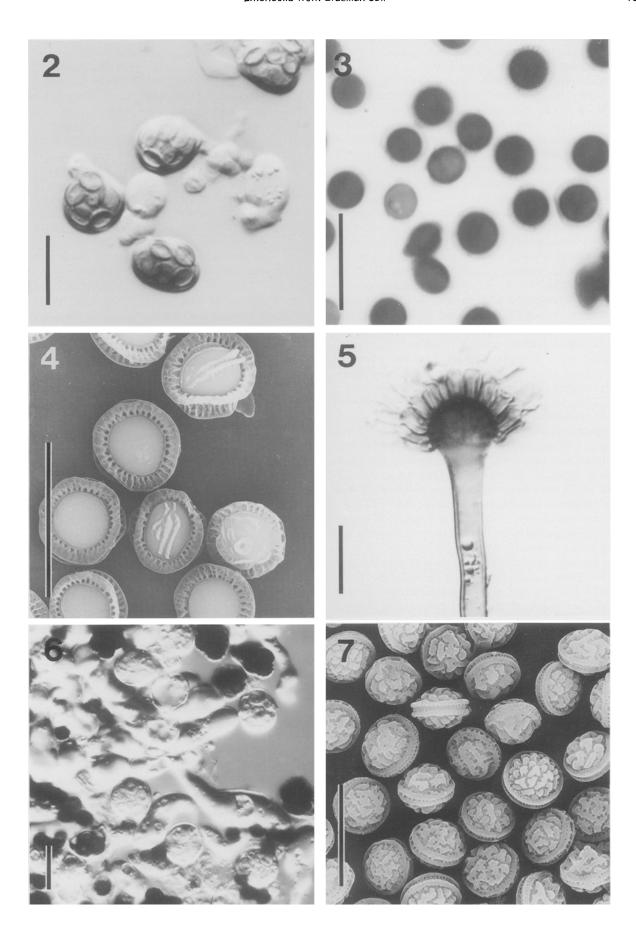
ing broadly, attaining a diameter of 83-85 mm in 14 d at 25°C, Pale Yellow (4A3) to Light Blond (4D3) or Greyish Green (30E7), consisting of a thin mycelial felt and loose aerial hyphae, floccose in appearance; ceistothecia abundantly produced; conidial heads abundantly produced; reverse Brownish Red (8C7) to Brownish Red (9C7).

Colonies on malt extract agar (MEA) spreading broadly, attaining a diameter of 80–85 mm in 14 d at 25°C, Pale Yellow (4A3) to Greyish Green (30E5), consisting of a thin mycelial felt; cleistothecia abundantly produced, granular in appearance, surrounded by a loose covering of hülle cells; conidial heads few in number; reverse Pale Yellow (4A3) to Greyish Yellow (4C3).

Cleistothecia superficial, reddish purple to dark reddish brown, globose to subglobose, 200-450 μ m in

Figs. 2-7. Emericella montenegroi and E. rugulosa var. lazulina.

^{2-5.} *E. montenegroi*; 2. Asci; 3. Ascospores; 4. Ascospores (SEM); 5. Aspergillum. 6, 7. *E. rugulosa* var. *lazulina*; 6. Asci; 7. Ascospores (SEM). All scales = 10 μ m.



diam, surrounded by yellowish white to yellow, globose to subglobose, thick-walled hülle cells measuring 13–20 μ m in diam. Peridium grayish yellow to dull red, membranaceous, 2–3-layered, consisting of angular cells measuring 4–19 μ m in diam. Asci 8-spored, subglobose to ovoid, 8.5– 12×7 –8 μ m, very evanescent. Ascospores at first hyaline to pale yellowish brown, then becoming dull red to reddish brown or grayish red at maturity, lenticular, spore bodies 3.6– 4.8×3.2 –3.6 μ m, provided with two equatorial crests measuring 1.0 μ m wide, with convex surfaces frequently incompletely reticulate or ribbed when viewed with SEM.

Conidial heads grayish olive green to dull green, short columnar, 65–110 μ m long, 35–55(–80) μ m wide. Conidiophores brownish orange to grayish brown, more or less sinuous, smooth, thick-walled, arising from the basal felt or aerial hyphae, up to 180 μ m long, 4–5 μ m in diam at the middle. Vesicles brownish orange to grayish yellow, hemispherical to flask-shaped, 8–11 μ m in diam with metulae covering the upper 2/3–1/3 surface. Aspergilla biseriate; metulae orange gray to reddish gray, 5–6×2–2.5 μ m; phialides reddish white to reddish gray, 6–7×2–2.5 μ m. Conidia hyaline to pale yellowish brown, grayish green in mass, globose to subglobose, minutely roughened to echinulate, 3–4 μ m in diam.

At 37°C, growth-rate better than at 25°C, and with increased production of cleistothecia.

Specimen examined: CBM-FA-0669 (holotype), a dried culture of an isolate from roadside soil from Pro-Verde, Botucatú, São Paulo, Brazil, collected and developed by Y. Horie, 21 July 1993 (isolate number 93-BS-1012-4), in the laboratory, Biosciences Institute, "Faculdade de Medicina, Universidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil. The type specimen is deposited in the Natural History Museum and Institute, Chiba, Japan (CBM).

The ascospores of this species are similar to those of *E. nidulans* (Eidam) Vuill., but there is incompletely reticulate or ribbed ornamentation on the convex walls, instead of the smooth walls of the latter (Raper and Fennell, 1965).

Emericella rugulosa (Thom & Raper) C. R. Benjamin var. lazulina Horie, Miyaji & Nishimura, var. nov.

Figs. 6-8

A typo differt ascosporis lazulinis.

Etymology: Latin, *lazulinus*=blue, referring to the color of ascospores.

Holotypus CBM-FA-0701, colonia exiccata in cultura ex solo, Pro-Verde, Botucatú, São Pauro, Brazil, 21. VII. 1993, a Y. Horie isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis, Chiba (CBM) conservata.

Anamorphosis: Aspergillus rugulovalvus Samson & W. Gams pro parte.

Colonies on MEA growing restrictedly, attaining a diameter of 20-25 mm in 14 d at 25°C, Greyish Green

(29E5 to 30E6), consisting of a thick mycelial felt; cleistothecia granular in appearance; conidial heads very abundantly produced; reverse Brown (7E5 to 7E7).

Cleistothecia dark grayish brown to dark magenta, globose to subglobose, $70\text{--}230\,\mu\text{m}$ in diam, surrounded by grayish orange to brownish orange, globose to subglobose hülle cells measuring $13\text{--}20\,\mu\text{m}$ in diam. Peridium grayish brown to brownish gray, membranaceous, consisting of irregular-shaped cells measuring $3\text{--}15\,\mu\text{m}$ in diam. Asci 8-spored, globose to subglobose, $(9\text{--})10\text{--}12\times8\text{--}10\,\mu\text{m}$. Ascospores grayish magenta to grayish violet, dark blue in mass, broadly lenticular, spore bodies $5\text{--}5.5\times4\text{--}4.5\,\mu\text{m}$, with two low equatorial crests measuring $0.5\,\mu\text{m}$ wide, with convex walls rugulose and cerebriform when viewed with SEM.

Conidial heads grayish green to deep green, short columnar, commonly up to 45–115 μ m long, 30–65 μ m wide. Conidiophores grayish yellow to orange gray, more or less sinuous, smooth, up to 175 μ m long, 5–6 μ m in diam at the middle. Vesicles grayish yellow to orange gray, hemispherical to flask-shaped, 10–14 μ m in diam, with metulae covering the upper 2/3–1/2 surface. Aspergilla biseriate; metulae yellowish gray, 4–7×2–3 μ m; phialides yellowish gray, 5–8×2–3 μ m. Conidia hyaline to pale yellowish brown, light olive to green in mass, globose to subglobose, echinulate, 3–3.5 μ m in diam.

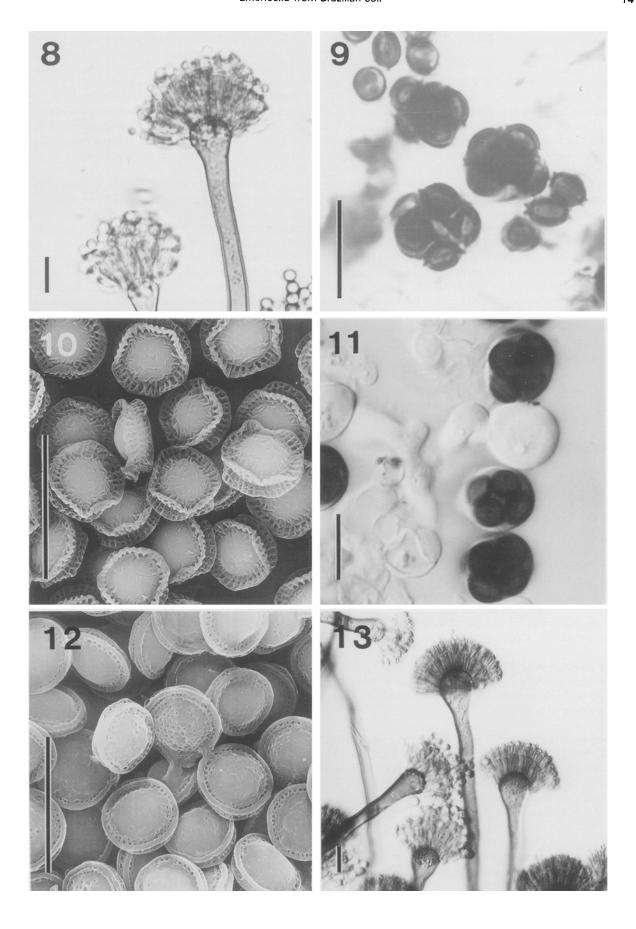
Colonies on Czapek agar growing restrictedly, attaining a diameter of 17-20 mm in 14 d at 25°C, Greyish Green (29E6 to 30E6), consisting of a thin mycelial felt, floccose; cleistothecia limited in number; conidial heads abundantly produced; reverse Brownish Orange (6C4) to Light Brown (6D5).

Colonies on CYA growing restrictedly, attaining a diameter of 21-25 mm in 14 d at 25°C, Greyish Green (30E4) to Greyish Ruby (12D3), consisting of a dense mycelial felt, furrowed; cleistothecia very abundantly produced, in a dense layer; conidial heads abundantly produced, particularly in marginal areas; reverse Brownish Orange (6D6) to Brown (6D7).

At 37°C, growth-rate better than at 25°C, and with increased production of cleistothecia.

Specimen examined: CBM-FA-0710 (holotype), a dried culture of an isolate from soil in a sugar plantation, Pro-Verde, Botucatú, São Paulo, Brazil, collected and developed by Y. Horie in the laboratory, Biosciences Institute, "Faculde de Medicina, Universidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil, 21 July 1993 (isolate number 93-BS-42). The type specimen is deposited in the Natural History Museum and Institute, Chiba, Japan (CBM).

Emericella rugulosa var. rugulosa frequently isolated from soil and herbal drugs, but ascospores are always in grayish red to dark grayish red or reddish purple (Benjamin, 1955; Raper and Fennell, 1965). The new variety differs from the type variety significantly only in the



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production of grayish magenta to grayish violet ascospores (Christensen and Raper, 1978; Horie, 1979, 1980).

Emericella corrugata Udagawa & Horie, Mycotaxon 4: 535. 1976. Figs. 9, 10

Colonies on MEA spreading broadly, attaining a diameter of 81-82 mm in 14 d at 25 °C, Light Yellow (4A4) to Greyish Yellow (4B6), consisting of a thin mycelial felt; cleistothecia very abundantly produced, granular in appearance; reverse Grey Orange (5B4 to 5B6). Anamorph not observed.

Cleistothecia violet brown to dark ruby, globose to subglobose, 130–290 μm in diam, surrounded by yellowish white to orange white, globose to subglobose hülle cells measuring 15–23 \times 15–20 μm . Peridium brownish orange to dark blond, consisting of angular to irregular-shaped cells measuring 3-13 μm in diam. Asci 8-spored, subglobose to ovoid, 8.5–11 \times 8–10 μm . Ascospores deep red to red or brownish red, broadly lenticular, spore bodies 4–4.5 \times 3.5–4 μm , with two equatorial crests measuring 0.5–0.7 μm wide, with convex walls corrugate when viewed with SEM.

Colonies on Czapek agar growing rapidly, attaining a diameter of 48-54 mm in 14 d at 25°C, Yellow White (4A2) to Greyish Yellow (4B3), consisting of a thin mycelial felt, floccose; cleistothecia few in number; reverse Light Brown (6D6) to Dark Brown (7F7). Anamorph not observed.

Colonies on CYA spreading broadly, attaining a diameter of 81–82 mm in 14 d at 25°C, Pale Yellow (4A3) to Greyish Brown (5E3), consisting of a thin mycelial felt; cleistothecia very abundantly produced in a dense layer; reverse Reddish Brown (8D7 to 8D8). Anamorph not observed.

At 37°C, growth-rate better than at 25°C, and with increased production of cleistothecia.

Specimen examined: CBM-FA-0687, an isolate from meadow soil, Carpina, Pernambuco, Brazil, collected and developed by Y. Horie in the "Laboratorio de Immunopathologia Keizo Asami, Campus de Universidade Federal de Pernambuco Cidade Universitaria," Recife, Pernambuco, Brazil, 7 June 1990 (isolate number 90-BP-169-1).

This species was first isolated from soil samples from a sugarcane field in Thailand (Udagawa and Horie, 1976). It is characterized by ascospores with corrugate convex surface (Fig. 10).

Emericella foveolata Horie, Trans. Mycol. Soc. Japan 19: 313. 1978. Figs. 11-13

St. Anam. Aspergillus foveolatus Horie, Trans. Mycol. Soc. Japan 19: 313. 1978.

Colonies on MEA spreading broadly, attaining a diameter of 80-81 mm in 14 d at 25°C, at first Light Yellow (4A4), then becoming Greyish Ruby (12D3) to Greyish Magenta (13E3), consisting of a thin mycelial felt; cleistothecia very abundantly produced, granular; conidial heads few in number; reverse Light Orange (5A4) to Greyish Brown (9D3).

Cleistothecia dark ruby, globose to subglobose, 120–530 μm in diam, surrounded by yellowish white, globose to ovoid hülle cells measuring 14–23 (–30) μm in diam. Peridium grayish orange to brownish orange, consisting of angular cells, measuring 3–13 μm in diam. Asci 8-spored, globose to ovoid, 10–12.5×9–10 μm . Ascospores grayish red to brownish violet or violet brown, broadly lenticular, spore bodies 4.5–5.5×4–4.5 μm , with two equatorial crests measuring 1.0 μm wide, with convex walls foveolate when viewed with SEM.

Conidial heads grayish green to dark green, short columnar, 48–80 μm long, 45–53 μm wide. Conidiophores grayish yellow to orange gray, more or less sinuous, smooth, up to 140 μm long, 4–6(–7) μm in diam at the middle. Vesicles grayish yellow to orange gray, hemispherical to flask-shaped, 8–16 μm in diam with metulae covering the upper 2/3–1/2 surface. Aspergilla biseriate; metulae 6–9 × 2.5–4 μm ; phialides 7–8 × 2.5–3 μm . Conidia hyaline to pale yellowish brown, grayish green to dull green in mass, globose to subglobose, minutely roughened, 3–4.5 μm in diam.

Colonies on Czapek agar spreading broadly, attaining a diameter of 71-73 mm in 14 d at 25°C, Yellow White (4A2) to Greyish Orange (5B3), consisting of a thin mycelial felt, more or less floccose; cleistothecia abundantly produced at central area, granular in appearance; conidial heads few in number; reverse Yellowish White (4A2) to Greyish Yellow (4B3).

Colonies on CYA spreading broadly, attaining a diameter of 85 mm with in 14 d at 25°C, Reddish White (8A2) to Brownish Grey (9C2), consisting of a thin mycelial felt; cleistothecia very abundantly produced in a dense layer; conidial heads few in number; reverse Orange (5A7).

At 37°C, growth-rate better than at 25°C, and with increased production of cleistothecia.

Specimen examined: CBM-FA-0686, an isolate from dung of armadillo, Pro-Verde, Botucatú, São Paulo State, Brazil, collected and developed by Y. Horie in the laboratory, Biosciences Institute, "Faculde de Medicina Universidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil, 21 July 1993 (isolate number 93-BS-8-1).

This species was first isolated from seed of an Indian herbal drug (*Tribulus terrestris*) (Horie, 1978). It is characterized by ascospores with foveolate convex walls (Fig. 12).

Synoptic key to the species

- 1. Emericella acristata (Fennell & Raper) Horie
- 2. Emericella astellata (Fennell & Raper) Horie
- 3. Emericella aurantio-brunnea (Atkins, Hinds & Russell) Malloch & Cain
- 4. Emericella bicolor Christensen & States
- 5. Emericella cleisto-minuta Mehrotra & Prasad
- 6. Emericella corrugata Udagawa & Horie
- 7. Emericella dentata (Sandhu & Sandhu) Horie
- 8. Emericella desertorum Samson & Mouchacca

- 9. Emericella echinulata (Fennell & Raper) Horie
- Emericella falconensis Horie, Miyaji, Nishimura & Udagawa
- 11. Emericella foeniculicola Udagawa
- 12. Emericella foveolata Horie
- Emericella fruticulosa (Raper & Fennell) Malloch & Cain
- Emericella heterothallica (Kwon, Fennell & Raper)
 Malloch & Cain
- 15. Emericella montenegroi Horie, Miyaji & Nishimura
- 16. Emericella navahoensis Christensen & States
- Emericella nidulans (Eidam) Vuill. var. lata Thom & Raper
- 18. Emericella nidulans (Eidam) Vuill. var. nidulans
- 19. Emericella omanensis Horie & Udagawa
- Emericella parvathecia (Raper & Fennell) Malloch & Cain
- 21. Emericella purpurea Samson & Mouchacca
- Emericella quadrilineata (Thom & Raper) C. R. Benjamin
- 23. Emericella rugulosa (Thom & Raper) C. R. Benjamin var. lazulina Horie, Miyaji & Nishimura
- 24. Emericella rugulosa (Thom & Raper) C. R. Benjamin var. rugulosa
- Emericella similis Horie, Udagawa, Abdullah & Al-Bader
- 26. Emericella spectabilis Christensen
- 27. Emericella striata (Rai, Tewari & Mukerji) Malloch &
- 28. Emericella sublata Horie
- 29. Emericella undulata Kong & Qi
- 30. Emericella unguis Malloch & Cain
- 31. Emericella variecolor Berk. & Br.
- 32. Emericella violacea (Fennell & Raper) Malloch & Cain
- 1. Colony color on MEA:

light gray to light yellowish gray.....1, 4, 6, 8, 17, 20, 21, 24, 25, 26, 27,

yellow to bright yellow.....3, 10, 13, 15, 16 brown to olivaceous green.....2, 5, 7, 9, 14, 18, 19,

23, 30, 31 reddish brown to grayish red·····11, 12, 22, 28, 29,

reddish brown to grayish red·····11, 12, 22, 28, 29, 32

2. Anamorph:

produced·····1, 2, 3, 4, 6, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24, 26, 27, 28, 29, 32

very abundantly produced.....5, 7, 9, 18, 19, 23, 30, 31

absent 6, 8, 25, 27, 32

- 3. Teleomorph:
- 1) Ascomatal formation:

homothallic·····1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32

heterothallic ······14

2) Ascospores:

Ascospore crests:

(a) two in number·····2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 23, 24, 25, 26, 28, 29, 30, 31, 32

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four in number·····1, 20, 22 dentate·····7 multiple·····27
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(b) narrow-moderate (up to 0.6 μ m wide).....1, 8, 11, 16, 20, 21, 22, 23, 24, 25, 27, 32, moderate (0.6–1.5 μ m wide).....3, 6, 9, 12, 13, 15, 18, 19, 26, 30

broad(up 1.5 μ m wide)······2, 10, 14, 17, 28 incised, stellate in pattern······29, 31

- (c) closely appressed.....1, 8, 11, 16, 21, 25, 27 separate or widely separate.....2, 3, 6, 9, 10, 12, 13, 14, 15, 17, 18, 19, 20, 22, 23, 24, 26, 28, 29, 31, 32
- (d) obscurely pleated……1, 8, 11, 16, 21, 23, 24, 25, 27, 32
 - distinctly pleated2, 3, 6, 9, 10, 12, 13, 14, 15, 17, 18, 19, 20, 22, 26, 28, 29, 31

Ascospore convex wall:

smooth······1, 2, 3, 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 18, 20, 21, 22, 26, 28, 30, 31

rugulose·····23, 24,

incompletely reticulate or ribbed 15

aculeate to tuberculate 8, 9, 19

capitate·····29
foveolate·····12
ribbed·····27
corrugate·····6
reticulate·····25, 32

Color of ascospores:

reddish purple to reddish brown·····1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 26, 27, 28, 29, 30, 31 blue to dark purplish brown·····23, 25, 32

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