## New and interesting species of *Neosartorya* from Brazilian soil

Yoshikazu Horie<sup>1)</sup>, Makoto Miyaji<sup>2)</sup>, Kazuko Nishimura<sup>2)</sup>, Marcello F. Franco<sup>3)</sup> and Kunie labuki R. Coelho<sup>3)</sup>

- <sup>1)</sup> Natural History Museum and Institute, Chiba, 955-2, Aoba-cho, Chuo-ku, Chiba-shi, Chiba 260, Japan
- 2) Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba-shi, Chiba 260, Japan
- <sup>3)</sup> Department of Pathology, School of Medicine, University of Estadual Paulista, Botucatú, São Paulo, Brazil

Accepted for publication 1 May 1995

Neosartorya udagawae, a new heterothallic species isolated from Brazilian soil, is described and illustrated. It is characterized by dull green colony on malt extract agar, light yellow large ascomata, broadly lenticular ascospores with two equatorial or several irregular crests and tuberculate convex surfaces, and with an Aspergillus anamorph. Neosartorya aureola is also described as a new record from Brazilian soil.

Key Words——Aspergillus aureoluteus; Aspergillus udagawae; Brazil; Neosartorya aureola; Neosartorya udagawae; soil fungi.

Most members of the genus *Neosartorya* Malloch & Cain (Malloch and Cain, 1972) in the Eurotiales are of worldwide distribution and are very abundant, occurring nearly everywhere in soil, air, house dust, food etc. (Domsch et al., 1980; Kozakiewicz, 1989, 1990; Udagawa et al., 1991; Yaguchi et al., 1994).

In the course of a study of thermophilic and thermotolerant fungi isolated from soils of a corn plantation and a sugarcane plantation in São Paulo State, Brazil, Neosartorya botucatensis, N. paulistensis and N. tatenoi were recently reported (Horie et al., 1992, 1995). Two additional isolates were obtained from the same samples: one is a new heterothallic species of Neosartorya, of which fertile cleistothecia were first observed in the primary isolation plate, where two mating strains were planted side by side; and the other is identified as N. aureola (Fennell & Raper) Malloch & Cain, a rare fungus known from Gold Coast and Liberia. Cultures of these species as well as dried materials are deposited at the Natural History Museum and Institute, Chiba (CBM) and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (IFM).

**Neosartorya udagawae** Horie, Miyaji & Nishimura, sp. nov. Figs. 1, 3-6

Fungus heterothallicus. Cleistothecia primo alba, postea flavo-alba vel laete flava, globosa vel subglobosa vel ovoidea,  $310\text{-}620\times280\text{-}530~\mu\text{m}$ , cum hyphis aeriis laxe intricatis circumdata; peridium tenue, cum cellulis angularibus  $2\text{-}12~\mu\text{m}$  diam constans. Asci octospori, globosi vel subglobosi,  $11\text{-}12\times9\text{-}12~\mu\text{m}$ , evanescentes. Ascosporae hyalinae vel dilute flavo-brunneae, late lenticulares, sine cristis  $5\text{-}5.5\times4\text{-}5~\mu\text{m}$ , duabus cristis aequatorialibus sed saepe irregularibus usque  $1~\mu\text{m}$  latis

praeditae, superficies convexae tuberculatae. Status anamorphus: Aspergillus udagawae.

Holotypus CBM-FA-0711, colonia exsiccata ex crusi CBM-FA-0702 × CBM-FA-0703 uterque ex solo in Lagoa Seka Avea, Botucatú, São Paulo State, Brazil, die 23 mense Aug. anno 1993, a Y. Horie isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis, Chiba (CBM) conservata. Isotypus IFM 46899.

Etymology: named as a memorial to Dr. Shun-ichi Udagawa, eminent mycologist.

Anamorphosis: Aspergillus udagawae Horie, Miyaji & Nishimura, anam. nov.

Capitula conidica columnaria. Conidiophora ex hyphis aeriis vel mycelio basali orientia; stipites hyalini vel dilute flavo-brunnei, usque 530  $\mu m$  longi, ad medium 4–6  $\mu m$  crassi, leves; vesiculae hemisphaericae vel ampulliformes, 12–15  $\mu m$  diam. Aspergilla uniserialia; phialides griseo-virides vel dilute virides, 5–7  $\times$  2–2.5  $\mu m$ . Conidia griseo-viridia vel dilute viridia, subglobosa vel late ellipsoidea, levia, 2.6–3.2  $\times$  2.4–2.6  $\mu m$ . Status teleomorphus: *Neosartorya udagawae*.

Coloniae in "CYA" celeriter crescentes, griseovirides vel herbeo-virides; conidiogenesis abundantia; reversum dilute aurantiacum vel laete aurantiacum.

Coloniae in "MEA" celeriter crescentes, albae vel flavo-albae vel laete flavae; conidiogenesis abundantia, dilute viridi; reversum laete aurantiacum vel griseo-aurantiacum.

Coloniae in "OA" celeriter crescentes, planae, viridigriseae vel herbeo-virides; conidiogenesis abundantia; reversum griseo-brunneum.

Holotypus CBM-FA-0702, colonia exsiccata ex solo in Lagoa Seka Avea, Botucatú, São Paulo State, Brazil, die 23 mense Aug. anno 1993, a Y. Horie isolata et ea col-

200 Y. Horie et al.

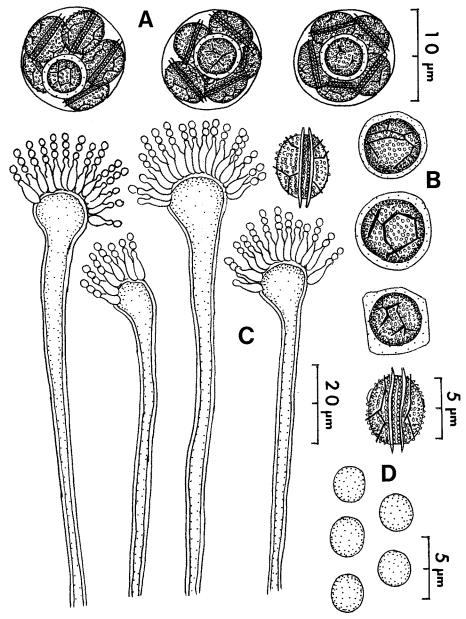


Fig. 1. Neosartorya udagawae.
A, Asci. B, Ascospores. C, Aspergilla. D, Conidia.

lectione fungorum, Musei et Instituti Historiae Naturalis, Chiba (CBM) conservata. Isotypus IFM 46868.

Heterothallic. Cleistothecia produced only when dissimilar mating types are paired, superficial, at first white, then becoming yellowish white to light yellow, globose to subglobose or ovoid,  $310\text{--}620\times280\text{--}530~\mu\text{m}$ , surrounded by a loose covering of hyaline to pale yellowish brown, 2–3  $\mu\text{m}$  wide aerial hyphae. Cleistothecial peridium hyaline to pale yellowish brown, thin, membranaceous, consisting of angular, 2–12  $\mu\text{m}$  diam cells. Asci 8-spored, globose to subglobose,  $11\text{--}12\times9\text{--}12~\mu\text{m}$ , evanescent at maturity. Ascospores hyaline to pale yellowish brown, broadly lenticular, spore body 5–5.5  $\times$  4–5  $\mu\text{m}$ , provided with two equatorial or often irregular crests up to 1  $\mu\text{m}$  wide; convex surfaces tuberculate (LM and SEM).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads graysih green to dull green, columnar, 95–145  $\times$  20–50  $\mu m$ . Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish brown, smooth, up to 530  $\mu m$  long, 4–6  $\mu m$  wide at the middle; vesicles hyaline to greenish, hemispherical to flask-shaped, 12–15  $\mu m$  in diam. Aspergilla uniseriate; phialides hyaline to greenish, covering the upper half of the vesicle, 5–7  $\times$  2–2.5  $\mu m$ . Conidia hyaline, grayish green to dull green in mass, subglobose to broadly ellipsoidal, smooth, 2.6–3.2  $\times$  2.4–2.6  $\mu m$ .

Colonies on CYA spreading broadly, attaining a diameter of 82-85 mm in 14 days at 25°C or 85 mm in 7 days at 37°C, Greyish Green (28D5, after Kornerup

and Wanscher, 1978) to Dull Green (27E4), floccose, consisting of a thin mycelial felt; conidial heads very abundantly produced; reverse Pale Orange (5A3) to Light Orange (5A6).

Colonies on MEA spreading broadly, attaining a diameter of 57-65 mm in 14 days at 25°C or 85 mm in 7 days at 37°C, Dull Green (27D3 to 29D3), floccose, consisting of a thin mycelial felt and loose aerial hyphae; conidial heads very abundantly produced; reverse Light Orange (5A4) to Greyish Orange (4B4).

Colonies on OA spreading broadly, attaining a di-

ameter of 83-85 mm in 14 days at 25°C or 85 mm in 7 days at 37°C, plane, Greenish Grey (30D2) to Dull Green (30D3 to 30E3), consisting of a thin mycelial felt; conidial heads abundantly produced; reverse Greyish Brown (9E3).

Specimen examined: CBM-FA-0711 (holotype), a dried colony from a paired culture of isolates CBM-FA-0702 (A) × CBM-FA-0703 (a) from soil in a plantation, Lagoa Seka Avea, Botucatú, São Paulo State, Brazil. Isolated and developed by Y. Horie in the laboratory, Biosciences Institute, "Faculdade de Medicina, Univer-

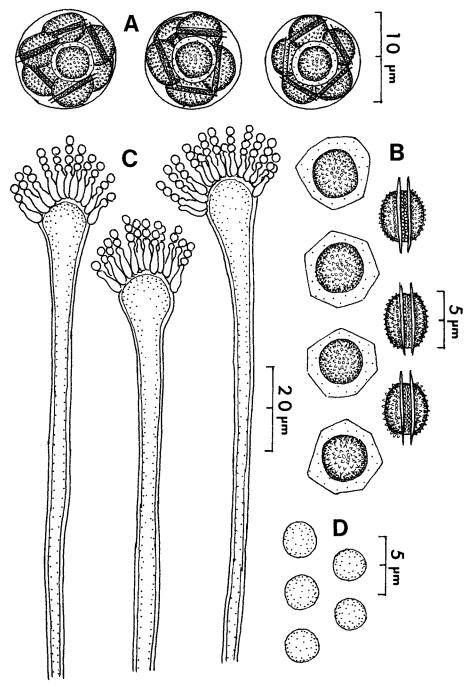


Fig. 2. Neosartorya aureola.

A, Asci. B, Ascospores. C, Aspergilla. D, Conidia.

202 Y. Horie et al.

sidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil, 23 August 1993. The type specimen is deposited in the Natural History Museum and Institute, Chiba, Japan (CBM). Isotype IFM 46868.

This heterothallic species can be recognized by its as-

cospores with two equatorial or often irregular, crests and tuberculate convex surfaces. *Neosartorya fennelliae* Kwon-Chung & Kim, the first heterothallic species, isolated from eye ball of rabbit (Kwon-Chung and Kim, 1974), is characterized by ascospores with two broad

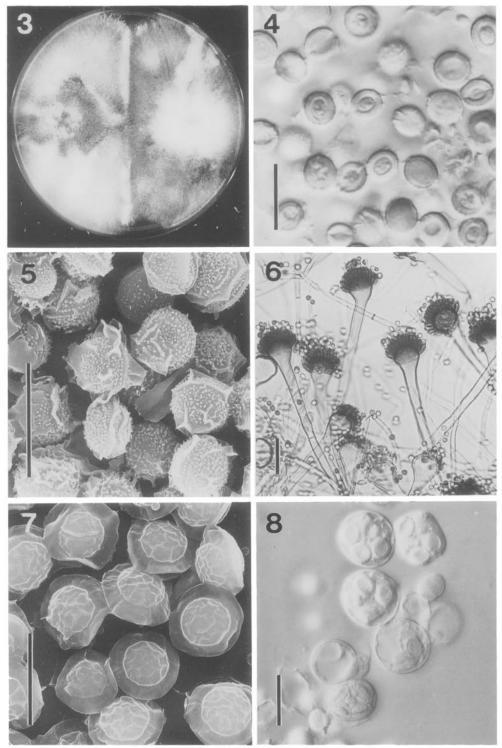


Fig. 3. Paired culture of *Neosartorya udagawae*, strain nos. CBM-FA-0702 $\times$ CBM-FA-0703, on MEA, 25°, 3 weeks. Fig. 4. Ascospores of *N. udagawae* (scale 10  $\mu$ m). Fig. 5. Ascospores of *N. udagawae*. SEM micrograph (scale 10  $\mu$ m). Fig. 6. Aspergilla of *N. udagawae* (scale 20  $\mu$ m). Fig. 7. Ascospores of *Neosartorya fennelliae*, strain nos. CBM-FA-0139 $\times$ CBM-FA-0140. SEM micrograph (scale 10  $\mu$ m). Fig. 8. Asci of *Neosartorya aureola* strain no., CBM-FA-0692 (scale 10  $\mu$ m).

equatorial crests and incompletely reticulate (cerebriform) surfaces (Fig. 7). The second heterothallic species, *N. spathulata* Takada & Udagawa, was isolated from Taiwan soil (Takada and Udagawa, 1985; Udagawa

and Takada, 1985). As with *N. fennelliae*, the ascospore ornamentation is the primary distinguishing feature: the ascospores of *N. spathulata* are characterized by two broad equatorial crests and nearly smooth to

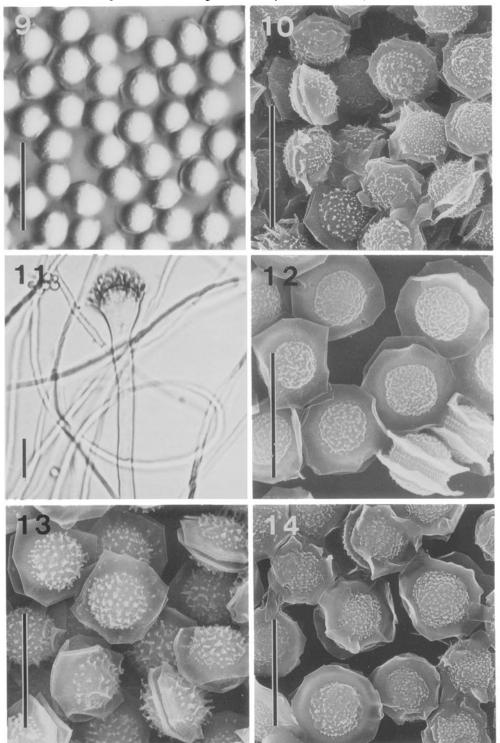


Fig. 9. Ascospores of Neosartorya aureola (scale  $10~\mu m$ ). Fig. 10. Ascospores of N. aureola. SEM micrograph (scale  $10~\mu m$ ). Fig. 11. Aspergillum of N. aureola (scale  $10~\mu m$ ). Fig. 12. Ascospores of Neosartorya glabra, strain no. CBM-FA-0158. SEM micrograph (scale  $10~\mu m$ ). Fig. 13. Ascospores of Neosartorya spinosa, strain no. CBM-FA-0168. SEM micrograph (scale  $10~\mu m$ ). Fig. 14. Ascospores of Neosartorya stramenia, strain no. CBM-FA-0173. SEM micrograph (scale  $10~\mu m$ ).

204 Y. Horie et al.

pustulate convex surfaces (Takada and Udagawa, 1985; Samson et al., 1990).

Neosartorya aureola (Fennell & Raper) Malloch & Cain, Can. J. Bot. 50: 2620. 1972; Kozakiewicz, Mycol. Pap. 161: 51. 1989. Figs. 2, 8-11 ≡ Aspergillus aureolus Fennell & Raper, Mycologia 47: 71. 1955.

St. Anam. Aspergillus aureoluteus Samson & W. Gams, Advances in *Penicillium* and *Aspergillus* systematics. p. 34. 1986.

Cleistothecia superficial, scattered, white to yellowish white, globose to ovoid, 350–500  $\mu m$  in diam, surrounded by a loose covering of yellow to orange, 2–4  $\mu m$  wide aerial hyphae. Cleistothecial peridium hyaline to pale yellowish brown, thin, consisting of irregular, 4–10  $\mu m$  diam cells. Asci 8-spored, globose to ovoid, 11–12×9.5–11  $\mu m$ , evanescent at maturity. Ascospores hyaline to pale yellowish brown, broadly lenticular, spore body 4–4.5×3.5–4  $\mu m$ , provided with two equatorial crests which are rather appressed, up to 1  $\mu m$  wide and rarely dissected, and with the convex surfaces aculeate with spines, up to 0.5  $\mu m$  long.

Mycelium hyaline to pale yellowish brown, branched, septate, smooth-walled. Conidial heads Greyish Green (28E5) to Dull Green (28D4), columnar to loose columnar,  $20\text{-}75\times20\text{-}50~\mu\text{m}$ . Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish brown, smooth, septate, up to 180  $\mu\text{m}$  long, 3-5  $\mu\text{m}$  wide at the middle. Vesicles hyaline to pale yellowish brown, flask-shaped, 8–13  $\mu\text{m}$  in diam. Aspergilla uniseriate; phialides pale yellowish brown, 3–7 × 2–3  $\mu\text{m}$ , covering the upper half of the vesicle. Conidia hyaline to pale yellowish brown, globose to subglobose or ovoid, smooth, 2.5–3.5 × 2.5–3  $\mu\text{m}$ .

Colonies on MEA spreading broadly, attaining a diameter of 75–76 mm in 14 days at 25 °C or 80–85 mm in 7 days at 37 °C, Pale Yellow (3A3) to Light Yellow (3A4), consisting of a thin, often submerged vegetative mycelium, characterized by abundant cleistothecia in a granular appearance or in small clusters, loosely overgrown by thin, yellow to orange aerial hyphae; conidial heads few in number; reverse Dark Brown (8F5).

Colonies on OA spreading broadly, attaining a diameter of 50–52 mm in 14 days at 25°C or 75–80 mm in 7 days at 37°C, Yellowish White to Pale Yellow (2A3), consisting of a thin mycelial felt, granular due to the abundant production of cleistothecia; details of aerial hyphae and conidial heads as on MEA; reverse Light Yellow (4A4) to Greyish Yellow (4B5).

Specimen examined: CBM-FA-0692 (=IFM 46584). In culture from the cultivated soil, Botucatú, São Paulo State, Brazil, isolated and developed by Y. Horie in the laboratory, Biosciences Institute, "Faculdade de Medicina, Universidade Estadual Paulista, Campus de Botucatú," São Paulo State, Brazil, 10 August 1993.

This species, first isolated from African soils in Gold Coast and Liberia (Fennell and Raper, 1955), is characterized by its rapid growth on common media, yellowish cleistothecia and aculeate to microaculeate convex sur-

face of ascospores.

Two species of *Neosartorya, N. aurata* (Warcup) Malloch & Cain and *N. stramenia* (Novak & Raper) Malloch & Cain, resemble this species in the yellow to golden pigmentation of loose aerial hyphae which envelop the cleistothecia. However, they differ in their very restricted growth on Czapek agar and smaller cleistothecia.

Differentiation based on the ascospore ornamentation between this species and the similar ones is also shown by SEM examination (Figs. 12-14). Detailed reports on the ascospore morphology of *Neosartorya* spp. have appeared elsewhere (Samson et al., 1990; Horie, 1992).

Acknowledgements——This work has been supported by a University-to-University Cooperative Research Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

## Literature cited

- Domsch, K. H., Gams, W. and Anderson, T.-H. 1980. "Compendium of soil fungi," vol. 1, pp. 511-512. Academic Press, London.
- Fennell, D. I. and Raper, K. B. 1955. New species and varieties of *Aspergillus*. Mycologia **47**: 68–89.
- Horie, Y. 1992. The genus *Neosartorya*. J. Antibact. Antifung. **20**: 27-34. (In Japanese.)
- Horie, Y., Miyaji, M., Yokoyama, K., Udagawa, S. and Campos-Takagi, G. M. 1992. *Neosartorya tatenoi*, a new species from Brazilian soil. Trans. Mycol. Soc. Japan **33**: 395– 399.
- Horie, Y., Miyaji, M., Nishimura, K., Franco, M. and labuki, K.R.C. 1995. Two new species of *Neosartorya* from Brazilian soil. Mycoscience **36**: 159-165.
- Kornerup, A. and Wanscher, J. H. 1978. "Methuen handbook of colour, 3rd ed.," Eyre Methuen, London. 252 p.
- Kozakiewicz, Z. 1989. Aspergillus species on stored products. CMI Mycological Papers 161: 1-188.
- Kozakiewicz, Z. 1990. Neosartorya fischeri. CMI Description of pathogenic fungi and bacteria, No. 1000. Mycopathologia 109: 201–202.
- Kwon-Chung, K. J. and Kim, S. J. 1974. A second heterothallic *Aspergillus*. Mycologia **66**: 628–638.
- Malloch, D. and Cain, R. F. 1972. The Trichocomataceae: Ascomycetes with Aspergillus, Paecilomyces, and Penicillium imperfect states. Can. J. Bot. 50: 2613-2628.
- Samson, R. A., Nielsen, P. V. and Frisvad, J. C. 1990. The genus Neosartorya: Differentiation by scanning electron microscopy and mycotoxin profiles. In: "Modern concepts in Penicillium and Aspergillus classification," (ed. by Samson, R. A. and Pitt, J. I.), pp. 455–467. Plenum Press, New York.
- Takada, M. and Udagawa, S. 1985. A new species of heterothallic *Neosartorya*. Mycotaxon **24**: 395-402.
- Udagawa, S. and Takada, M. 1985. Contribution to our knowledge of *Aspergillus* teleomorphs: some taxonomic problems. In: "Advances in *Penicillium* and *Aspergillus* systematics," (ed. by Samson, R. A. and Pitt, J. I.), pp. 429–435. Plenum Press, New York.
- Udagawa, S., Tsubouchi, H. and Horie, Y. 1991. *Neosartorya hiratsukae*, a new species of food-borne Ascomycetes. Trans. Mycol. Soc. Japan **32**: 23-29.
- Yaguchi, T., Someya, A. and Udagawa, S. 1994. A new species of *Neosartorya* from Taiwan soil. Mycoscience **35**: 309-313.