## Two new species of *Neosartorya* from Brazilian soil

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Among the isolates from soil of corn and sugar cane plantations in São Paulo State, Brazil, two new species of *Neosartorya, N. botucatensis* and *N. paulistensis*, are described and illustrated. *Neosartorya botucatensis* differs from the other known species of the genus in having ascospores with long spines on the convex walls. *Neosartorya paulistensis* is characterized by its ascospore walls with spinose and verruculose ornamentation. The former is compared with the closely related species *N. spinosa*, and the latter is closely related to *N. glabra, N. pseudofischeri and N. stramenia*.

Key Words—Aspergillus botucatensis; Aspergillus paulistensis; Brazil; Neosartorya botucatensis; Neosartorya paulistensis; soil fungi.

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

In the course of the study of thermophilic and thermotolerant fungi on soil in Brazil, two cleistothecial ascomycetes belonging to *Neosartorya* were isolated. These species sufficiently differ from all described species of the genus (Samson et al., 1990; Horie et al., 1992) to warrant ascribing them as a new species. Living cultures of these species as well as the dried specimens are deposited at the Natural History Museum and Institute, Chiba (CBM) and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (IFM).

## Neosartorya botucatensis Horie, Miyaji & Nishimura, sp. nov. Figs. 1, 3-7

Coloniae in "MEA (malt extract agar)" celeriter crescentes, flavo-albae vel aurantio-albae, granulares; cleistothecia abundantia; conidiogenesis limitata, dilute viridia vel griseo-viridia; reversum dilute flavum vel laete flavum. Coloniae in "OA (oatmeal agar)" celeriter crescentes, planae, albae vel flavo-albae, granulares; cleistothecia abundantia sed conidia limitata; reversum flavo-album.

Cleistothecia alba vel flavo-alba, globosa vel subglobosa, vulgo 160-340  $\mu$ m diam, cum hyphis aeriis laxe intricatis circumdata; peridium tenue, cum cellulis angularibus 4-13  $\mu$ m latis compositum. Asci octospori, globosi vel subglobosi vel ovoidei, 12-13 × 10-12  $\mu$ m, evanescentes. Ascosporae hyalinae vel dilute flavobrunneae, late lenticulares, sine cristis 4-4.5 × 3.54.5  $\mu$ m, duabus cristis aequatorialibus usque 2  $\mu$ m latis praeditae, cum paginis longispinosae, usque 5 (-7)  $\mu$ m longae. Status anamorphus: *Aspergillus botucatensis*.

Holotypus CBM-FA-0672, colonia exsiccata ex solo in Botucatu, São Paulo, Brazil, die 12 mense Aug. anno 1993, a Y. Horie isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis, Chiba (CBM) conservata. Isotypus IFM 46586.

Etymology: Lat. *botucatensis*, referring to Botucatu, the type locality.

Anamorphosis: *Aspergillus botucatensis* Horie, Miyaji & Nishimura, anam. nov.

Capitula conidica columnaria; stipites hyalini vel dilute flavo-brunnei, usque 490  $\mu$ m longi, ad medium 4-7  $\mu$ m crassi, leves; vesiculae hemisphaericae vel ampulliformes, 11-12  $\mu$ m crassae. Aspergilla uniserialia; phialides 7-8×2.5-3  $\mu$ m. Conidia dilute griseo-viridia, subglobosa vel late elliptica, 2-3.5×2-3  $\mu$ m, levia. Status teleomorphus: *Neosartorya botucatensis*.

Holotypus CBM-FA-0672, loc. cit.

Colonies on MEA spreading broadly, attaining a diameter of 85 mm within 14 days at 25°C or 85 mm within 7 days at 37°C, Yellowish White (4A2, after Kornerup and Wanscher, 1978) to Orange White (5A2), with a thin, often submerged vegetative mycelium, characterized by very abundant cleistothecia of granular appearance, loosely overgrown by aerial hyphae; conidial heads few in number, Pale Green (30C3) or Greyish Green(28C3); reverse Pale Yellow (4A3) to Light Yellow (4A4).

Colonies on OA spreading broadly, attaining a diameter of 85 mm within 14 days at  $25^{\circ}$ C or 69-72 mm within 7 days at  $37^{\circ}$ C, plane, White to Yellowish White (4A2), consisting of a thin mycelial felt, granular due to



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

the abundant production of cleistothecia in a loose to dense layer, loosely overgrown by aerial hyphae; conidial heads few in number, scattered; reverse Yellowish White (4A2).

Cleistothecia superficial, white to yellowish white, globose to subglobose, 160-340  $\mu$ m in diam, surrounded by a loose covering of hyaline to pale yellowish brown, 2-

3  $\mu$ m wide hyphae. Cleistothecial peridium hyaline to pale yellowish brown, membranaceous, consisting of angular, 4-13  $\mu$ m diam cells. Asci 8-spored, globose or subglobose to ovoid, 12-13×10-12  $\mu$ m, evanescent at maturity. Ascospores hyaline to pale yellowish brown, broadly lenticular, spore body 4-4.5×3.5-4.5  $\mu$ m, provided with two well-separated equatorial crests which Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads short columnar to columnar,  $50-80 \times 40-75 \ \mu m$ . Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish brown, smooth, more or less sinuous, up to 490  $\mu m$  long, 4-7  $\mu m$  wide at the middle; vesicles hyaline to more or less greenish, hemispherical to flask-shaped, 11-12  $\mu$ m in diam. Aspergilla uniseriate; phialides hyaline to more or less greenish, 7-8×2.5-3  $\mu$ m, covering the upper half of vesicle. Conidia pale grayish green, subglobose to broadly elliptical, smooth, 2-3.5×2-3  $\mu$ m.

At 37°C, growth is more rapid than at 25°C.

Specimen examined: CBM-FA-0672 (holotype), a dried culture of an isolate from soil in a corn plantation, Botucatú, São Paulo State, Brazil, collected and developed in the laboratory by Biosciences Institute, "Faculdade de Medicina, Universidade Estadual Paulista, Campus de Botucatú," São Paulo, Brazil, by Horie, 12 August



Fig. 2. Neosartorya paulistensis. A, Asci. B, Ascospores. C, Aspergilla. D, Conidia.

1993 (isolate number 93-BS-1168-1). The isotype has been deposited in the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, as IFM 46586.

This species is characterized by ascospores with long spinose convex surfaces. It is similar to *N. spinosa* 



Fig. 3. Asci of *Neosartorya botucatensis* (scale  $10 \,\mu$ m). Fig. 4. Ascospores of *N. botucatensis* (scale  $10 \,\mu$ m). Fig. 5. Ascospores of *N. botucatensis*. SEM photograph (scale  $10 \,\mu$ m). Fig. 6. Aspergillum of *N. botucatensis* (scale  $10 \,\mu$ m). Fig. 7. Conidia of *N. botucatensis* (scale  $10 \,\mu$ m). Fig. 8. Ascospores of *Neosartorya spinosa*. SEM photograph (scale  $10 \,\mu$ m).

(Raper & Fennell) Kozak., although ascospore ornamentation of the latter is short aculeate as shown in Fig. 8. Neosartorya paulistensis Horie, Miyaji & Nishimura, sp. nov. Figs. 2, 9-13 Coloniae in MEA celeriter crescentes, floccosae, pla-



Fig. 9. Asci of *Neosartorya paulistensis* (scale  $10 \,\mu$ m). Fig. 10. Ascospores of *N. paulistensis* (scale  $10 \,\mu$ m). Fig. 11. Ascospores of *N. paulistensis*. SEM photograph (scale  $10 \,\mu$ m), Fig. 12. Aspergillum of *N. paulistensis* (scale  $10 \,\mu$ m). Fig. 13. Conidia of *N. paulistensis* (scale  $10 \,\mu$ m). Fig. 14. Ascospores of *Neosartorya pseudofischeri*. SEM photograph (scale  $10 \,\mu$ m).

nae, albae vel viridi-griseae; cleistothecia abundantia sed conidia limitata; reversum dilute flavum. Coloniae in OA celeriter crescentes, floccosae vel funiculosae, albae; cleistothecia abundantia sed conidia limitata; reversum flavo-album.

Cleistothecia alba vel flavo-alba, globosa vel subglobosa, vulgo 360-750  $\mu$ m diam, cum hyphis aeriis laxe intricatis circumdata; peridium tenue, cum cellulis angularibus 4-15  $\mu$ m latis compositum. Asci octospori, globosi vel subglobosi vel ovoidei, 10-12.5×10-11.5  $\mu$ m, evanescentes. Ascosporae hyalinae vel dilute flavo-brunneae, late lenticulares, sine cristis 4-5.5×4-4.5  $\mu$ m, dubus cristis aequatorialibus 1  $\mu$ m latis praeditae, verrucosae et spinosae, cum spinis usque 1  $\mu$ m longae. Status anamorphus: *Aspergillus paulístensis*.

Holotypus CBM-FA-0690, colonia exsiccata ex solo in Vitoriana, Botucatú, São Paulo, Brazil, die 10 mense Aug. anna 1993, a Y. Horie isolata et ea collectione fungorum, Musei et Instituti Historiae Naturalis, Chiba, conservata. Isotypus IFM 46585.

Etymology: Lat. *paulistensis*, referring to Paulista (São Paulo), the type locality.

Anamorphosis: *Aspergillus paulistensis* Horie, Miyaji & Nishimura, anam. nov.

Capitula conidica dilute viridia vel griseo-viridia, columnaria; stipites hyalini vel dilute flavo-brunnei usque 420  $\mu$ m longi, ad medium 4-8  $\mu$ m crassi, leves; vesiculae hemisphaericae vel ampulliformes, 11-14  $\mu$ m crassae. Aspergilla uniserialia; phialides 6-8 × 1.5-2  $\mu$ m. Conidia hyalina vel dilute flavo-brunnea, globosa vel subglobosa, 2-3  $\mu$ m diam.

Holotypus CBM-FA-0690, loc. cit.

Colonies on MEA spreading broadly, attaining a diameter of 85 mm within 14 days at 25°C or 82 mm within 7 days at 37°C, White to Greenish Grey (30B2), consisting of a thin mycelial felt and loose aerial hyphae, floccose; cleistothecia very abundantly produced of loose layer; conidial heads few in number, scattered, Pale Green (30C3) or Greyish Green (28C3); reverse Pale Yellow (4A3).

Colonies on OA spreading broadly, attaining a diameter of 85 mm within 14 days at 25°C or 73-78 mm within 7 days at 37°C, White, consisting of a thin mycelial felt and loose aerial hyphae, floccose to more or less funiculose; cleistothecia very abundantly produced, overgrown by aerial hyphae; conidial heads few in number, scattered in small group, Greysih Green(28C3) or Dull Green(23D3); reverse Yellowish White (4A2).

Cleistothecia superficial, white to yellowish white, globose to subglobose, mostly 360-750  $\mu$ m in diam, surrounded by a loose covering of hyaline to pale yellowish brown, 2-5  $\mu$ m wide hyphae. Cleistothecial peridium hyaline to pale yellowish brown, membranaceous, thin, consisting of angular, 4-15  $\mu$ m diam cells. Asci 8spored, globose to subglobose or ovoid, 10-12.5×10-11.5  $\mu$ m, evanescent at maturity. Ascospores hyaline to pale yellowish brown, broadly lenticular, spore body 4.5-5.5×4-4.5  $\mu$ m, provided with two widely separated equatorial crests up to 1  $\mu$ m wide, with convex surfaces irregularly roughened by verruculose and small triangular projections up to 1  $\mu$ m long (as shown in Figs. 10, 11).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads loosely columnar,  $35-80 \times 25-50 \ \mu\text{m}$ . Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish brown, smooth, more or less sinuous, up to 420  $\mu$ m long, 4-8  $\mu$ m wide at the middle; vesicles hyaline to more or less greenish, hemispherical to flask-shaped, 11-14  $\mu$ m in diam. Aspergilla uniseriate; phialides hyaline to pale olive,  $6-8 \times 1.5-2 \ \mu$ m, covering the upper half of vesicle. Conidia hyaline to pale yellowish brown, globose to subglobose, smooth, 2-3  $\mu$ m in diam.

At 37°C, growth is more rapid than at 25°C.

Specimen examined: CBM-FA-0690 (holotype), a dried culture of an isolate from soil in a sugar cane plantation, Botucatu, São Paulo State, Brazil, collected and developed in the laboratory by Biosciences Institute, "Faculdade de Medicina. Universidade Estadual Paulista, Cam-



Fig. 15. Ascospores of Neosartorya glabra. SEM photograph (scale 10 μm). Fig. 16. Ascospores of Neosartorya stramenia. SEM photograph (scale 10 μm).

pus de Botucatú," São Paulo, Brazil by Y. Horie, 10 August 1993 (isolate number 93-BS-587). The isotype has been deposited in the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, as IFM 46585.

This species is characterized by ascospores with very widely separated equatorial crests, and verruculose and small triangular projections. Ascospores of *N. paulistensis* are similar to those of *N. glabra* (Fennell & Raper) Kozak., *N. pseudofischeri* Feterson and *N. stramenia* (Novak & Raper) Malloch & Cain (Raper and Fennell, 1965; Kozakiewicz, 1989; Peterson, 1992). The ornamentation of ascospore convex surfaces of *N. glabra* is microtuberculate (Fig. 15). The convex surfaces of ascospores of *N. pseudofischeri* are strongly and irregularly ribbed (Fig. 14). The ascospores of *N. glabra* and *N. pseudofischeri*, but the ascoma color is distinctly yellowish.

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