

FULL PAPER

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Two new species of *Neosartorya* from Amazonian soil, Brazil

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Abstract *Neosartorya indohii* and *N. tsurutae*, two new *Neosartorya* species isolated from tropical rainforest soil in the Amazonian area, Brazil, are described and illustrated. *Neosartorya indohii* is characterized by its spreading growth on Czapek's and malt extract agars, light yellow cleistothecia, broadly lenticular ascospores with two conspicuously serrate-incised equatorial crests and tuberculate convex surfaces, and globose to subglobose conidia with a smooth wall. *Neosartorya tsurutae* is characterized by its spreading growth on Czapek's and malt extract agars, white cleistothecia, broadly lenticular ascospores with four equatorial crests and rugulose-ruminant convex surfaces, and ovoid to broadly ellipsoidal conidia with a smooth wall.

Key words Amazon · *Aspergillus indohii* · *Aspergillus tsurutae* · *Neosartorya indohii* · *Neosartorya tsurutae*

Introduction

In a survey of pathogenic and mycotoxin-producing fungi in Brazil during the last decade, uncommon isolates of *Neosartorya* Malloch & Cain have been collected from soil of tropical rain forest in the Amazon area of Brazil. The first isolate, 01-BA-193-1, is characterized by production of

broadly lenticular ascospores with two conspicuously serrate equatorial crests and tuberculate surfaces on the convex walls. The second isolate, 01-BA-739-2, is characterized by production of broadly lenticular ascospores with four equatorial crests and rugulose surfaces on the convex walls. These isolates proved to be sufficiently different from all described species of *Neosartorya* (Horie et al. 1992, 1995a,b, 2001; Kong 1997; Kozakiewicz 1989; Kwon-Chung and Kim 1974; Malloch and Cain 1972; Peterson 1992; Raper and Fennell 1965; Samson et al. 1990; Someya et al. 1999; Takada and Udagawa 1985; Takada et al. 2001; Udagawa and Takada 1985; Udagawa et al. 1991, 1993; Yaguchi et al. 1994). These fungi are described herein. A key to all accepted species of the genus is provided.

Material and methods

Isolation of fungi

We surveyed 685 Brazilian soil samples. The soil collecting areas were 11 locations in Brazil, Cruzeliro do Sul (Acre state), Porto Acre (Acre st.), Xapuri (Acre st.), Rio Branco (Acre st.), Boca do Acre (Amazonas st.), Manaus (Amazonas st.), Port Velho (Rondonia st.), Alto Paraiso (Rondonia st.), Jaci-Parana (Rondonia st.), Rio Fomosa (Pernambuco st.), and Carnauda dos Dontos (Rio Grande do North st.).

The Aspergilli were isolated by a soil plate method of Czapek's agar, with incubation at 37°C for 10 days. *Neosartorya* spp. were obtained from 110 soil samples: *Neosartorya aureola* (Fennell & Raper) Malloch & Cain, *N. botucatensis* Horie, Miyaji & Nishimura, *N. fischeri* (Wehmer) Malloch & Cain, *N. glabra* (Fennell & Raper) Kozak., *N. pseudofischeri* S.W. Peterson, *N. quadricincta* (E. Yuill) Malloch & Cain, *N. spinosa* (Raper & Fennell) Kozak., *N. tatenoi* Horie, Miyaji, Yokoyama, Udagawa & Takagi, and two undescribed species of *Neosartorya*.

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Incubation and observation

Cultures were grown in a 25°C incubator room or in an incubator at 37°C. Fungal structure of the isolates, grown for 7–21 days on Czapek's (CzA), malt extract (MEA), and oatmeal (OA) agars, was examined by light and scanning electron microscopes (SEM). Colony colors are as designated in the Methuen Handbook of Colour (Kornerup and Wanscher 1978).

Deposition of fungal materials

The type specimen studied and living cultures derived from the type are deposited at the Natural History Museum and Institute Chiba (CBM) and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (IFM).

Taxonomy

Neosartorya indohii Y. Horie, sp. nov. Figs. 1, 3–6

Cleistothecia laete flava, globosa vel subglobosa, 195–325 µm diam., cum hyphis aeriis laxe intricatis circumdata; peridium tenue, membranaceum, ex cellulis angularibus 3–10 µm diam. compositum. Asci octospori, globosi vel subglobosi, 10–12.5 µm diam., evanescentes. Ascosporae hyalinae vel dilute flavo-brunneae, late lenticulares, praeter cristam 4–5.5 × 4–5 µm, duabus cristis aequatorialibus serratis vel inci sis usque 2.0 µm latis praeditae, superficie convexa tuberculata. Status anamorphus: *Aspergillus indohii*.

Holotypus: CBM-FA-934, colonia exsiccata ex solo in Cruzeiro do Sul, Acre st., in Brasilia, Nov. 3, 2001, a Y. Horie isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis Chiba (CBM) conservata. Isotypus: IFM 52113

Etymology: Named in memory of the late honorary Professor Hiroharu Indoh, Tokyo University of Education (now University of Tsukuba), eminent mycologist, whose research contributed so much to the Zygomycetes.

Anamorphosis: *Aspergillus indohii* Y. Horie, anam. nov.

Capitula conidica griseo-viridia, radiantia, 85–130 µm diam. Conidiophora ex hyphis aeriis vel mycelio basali orientia; stipites hyalini vel dilute flavo-brunnei, leves, usque 80 µm longi, in medio 4.5–5.5 crassi; vesiculae hyalinae vel dilute griseo-virides, ampulliformes, 9–13 µm diam. Aspergilla uniserialia; phialides hyalinae, ampulliformes, 5–7 × 2–3 µm, in summa 1/2 vesiculae obducentes. Conidia hyalina vel dilute griseo-viridia, globosa vel subglobosa, 2.5–3.5 µm diam., levia. Status teleomorphus: *Neosartorya indohii*.

Coloniae in CzA celeriter crescentes, albae vel griseo-flavae vel flavo-griseae, floccosae; cleistothecia et conidiogenesis limitata; reversum aurantio-griseum vel laete brunneum.

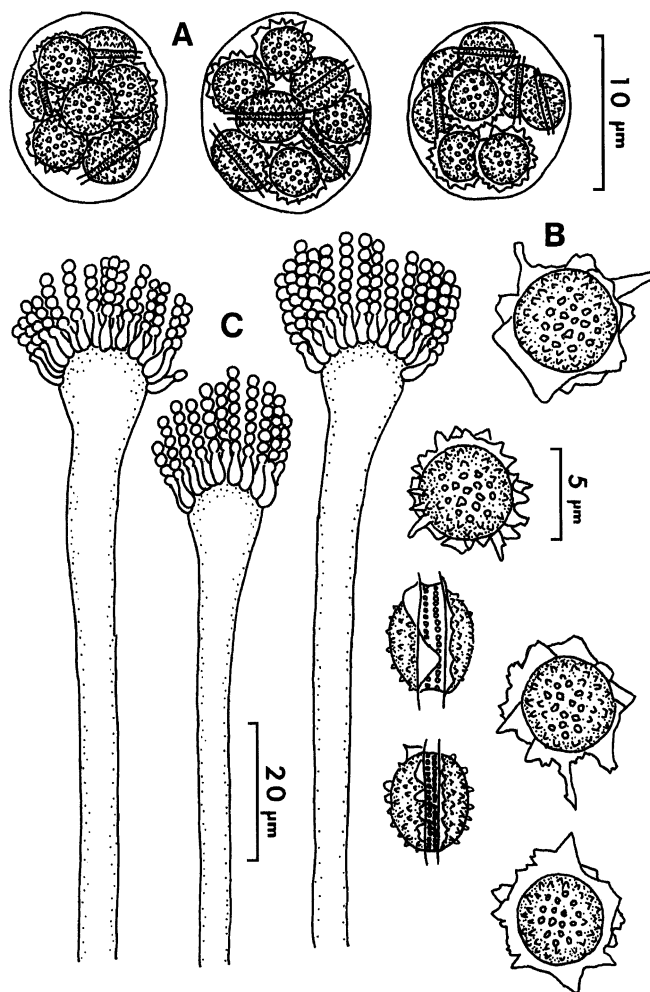


Fig. 1. *Neosartorya indohii*. A Asci. B Ascospores. C Aspergilla

Coloniae in MEA celeriter crescentes, albae vel laete flavae, granulares; cleistothecia abundantia; conidiogenesis limitata; reversum dilute flavum vel laete flavum.

Coloniae in OA celeriter crescentes, planae, dilute flavae vel flavo-albae, granulares; cleistothecia abundantia; conidiogenesis limitata; reversum flavo-album vel laete flavum.

Cleistothecia superficial, scattered or in small groups, light yellow, globose to subglobose, 195–325 µm in diameter, surrounded by a loose covering of aerial hyphae; peridium pale yellowish-brown, thin, membranaceous, consisting of angular cells, 3–10 µm in diameter. Asci 8-spored, globose to subglobose, 10–12.5 µm in diameter, evanescent at maturity. Ascospores hyaline to pale yellowish-brown, broadly lenticular, spore body 4–5.5 × 4–5 µm, provided with two conspicuously serrate to incised equatorial crests that are up to 2.0 µm wide, with convex surfaces tuberculata (SEM).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads greyish-green, radiate, 85–130 µm in diameter. Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish-brown, smooth, up to 80 µm long, 4.5–5.5 µm wide

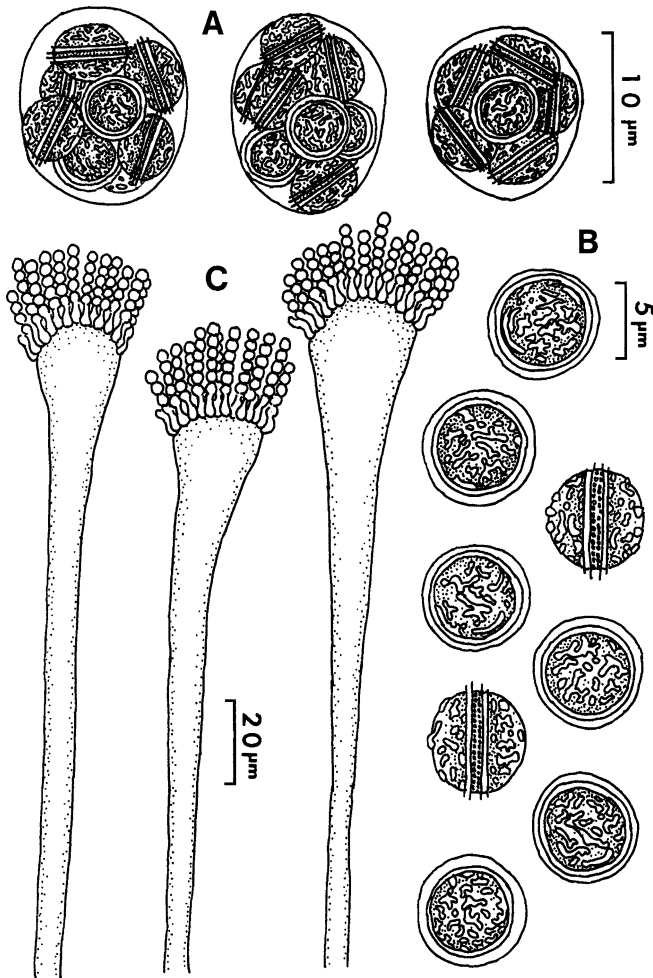


Fig. 2. *Neosartorya tsurutae*. A Asci. B Ascospores. C Aspergilla

at the middle; vesicles hyaline to pale greyish-green, flask-shaped, 9–13 µm in diameter. Aspergilla uniseriate; phialides hyaline, flask-shaped, 5–7 × 2–3 µm, covering upper half of the vesicle. Conidia hyaline to pale greyish-green, globose to subglobose, 2.5–3.5 µm in diameter, smooth.

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

Specimen examined: CBM-FA-934 (holotype), a dried culture of an isolate from soil in a grassland in a tropical rain forest, Cruzeiro do Sul, Acre state, Brazil, Nov. 3, 2001, collected and developed in the laboratory of Universidade Católica for Pernambuco, Recife, Pernambuco st., Brazil, by Y. Horie, Nov. 3, 2001 (isolate number 01-BA-193-1). Isotype: IFM 52113.

Colonies on CzA spreading broadly, attaining a diameter of 79–80 mm in 14 days at 25°C, white to greyish-yellow (4B3) or yellowish-grey (5D4), floccose, consisting of a thin mycelial felt; cleistothecia and conidia few in number; reverse orange-grey to light brown.

Colonies on MYA spreading broadly, attaining a diameter of 8.5 mm within 14 days at 25°C, white to light yellow (4A4), with a thin, often submerged vegetative mycelium, granular in appearance, due to the very abundant produc-

tion of cleistothecia; conidiogenesis few in number; reverse pale yellow (4A3) to light yellow (3A5).

Colonies on OA spreading broadly, attaining a diameter of 85 mm within 14 days at 25°C, plane, pale yellow (2A3) to yellowish-white (3A2), consisting of a thin mycelial felt, granular due to the abundant production of cleistothecia in a loose or dense layer, loosely overgrown by aerial hyphae; conidiogenesis few in number; reverse yellowish-white to light yellow.

This fungus is readily distinguished from the other species of *Neosartorya* by two conspicuously serrate or incised equatorial crests, which are up to 2 µm, and tuberculate convex surfaces.

Neosartorya tsurutae Y. Horie, sp. nov. Figs. 2, 7–10

Cleistothecia alba vel flavo-alba, globosa vel subglobosa, 150–250 µm diam., cum hyphis aeriis laxe intricatis circumdata; peridium tenue, membranaceum, ex cellulis angularibus 4–13 µm diam. compositum. Asci octospori, globosi vel subglobosi vel ovoidei, 9.5–12 × 11.5–14.5 µm, evanescentes. Ascospores hyalinae vel dilute flavo-brunneae, late lenticulares, praeter cristam 5.5–6.5 × 4–5.5 µm, quatuor cristis aequatorialibus usque 0.5–0.8 µm latis praeditae, superficie convexa rugulosa vel ruminata. Status anamorphus: *Aspergillus tsurutae*.

Holotypus: CBM-FA-933, colonia exsiccata ex solo in Rio Urubu, Urbs President Figueiredo, Amazonas st., in Brasilia (100 km north from Manaus), Nov. 12, 2001, a Y. Horie isolate et ea collectione fungorum Musei et Instituti Historiae Naturalis Chiba (CBM) conservata. Isotype: IFM 52114.

Etymology: Named in memory of the late Dr. Osamu Tsuruta, National Food Research Institute, eminent mycologist, whose research contributed so much to the study of foodborne and mycotoxin-producing fungi.

Anamorphosis: *Aspergillus tsurutae* Y. Horie, anam. nov.

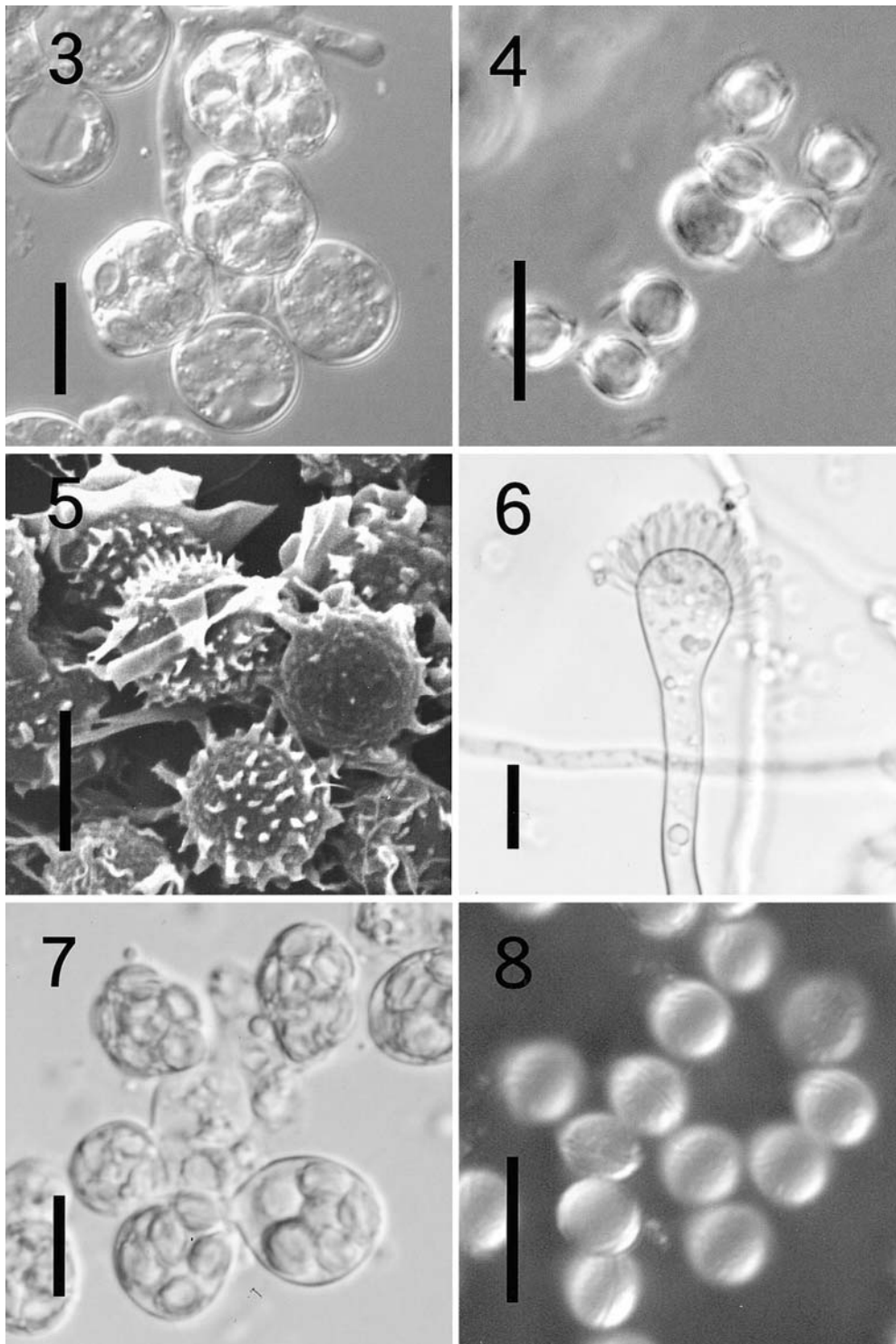
Capitula conidica griseo-viridia, radiantia vel brevicolumnaria, 80–165 × 10–20 µm. Conidiophora ex hyphis aeriis vel mycelio basali orientia; stipites hyalini vel dilute flavo-brunnei, leves, usque 735 µm longi, in medio 6–10 µm crassi; vesiculae hyalinae, hemisphaericae vel ampulliformes, 20–30 µm diam. Aspergilla uniseriata; phialides hyalinae vel olivaceo-griseae, 5–7 × 1.5–2.5 µm, in summa 1/2 vesicula obducentes. Conidia hyalina vel dilute griseo-iridia, ovoidea vel late ellipsoidea, 2.5–3.5 × 2.5 µm, levia. Status teleomorphus: *Neosartorya tsurutae*.

Coloniae in CzA celeriter crescentes, olivaceo-albae vel griseo-brunneae, zonatae; cleistothecia limitata; conidiogenesis abundantia; reversum flavo-album vel griseo-flavum.

Coloniae in MEA celeriter crescentes, albae vel flavo-griseae vel griseo-irides, granulares; cleistothecia abundantia; conidiogenesis abundantia; reversum flavo-album vel dilute flavum.

Coloniae in OA celeriter crescentes, aurantiaceo-albae, cleistothecia abundantia; conidiogenesis limitata; reversum flavo-album.

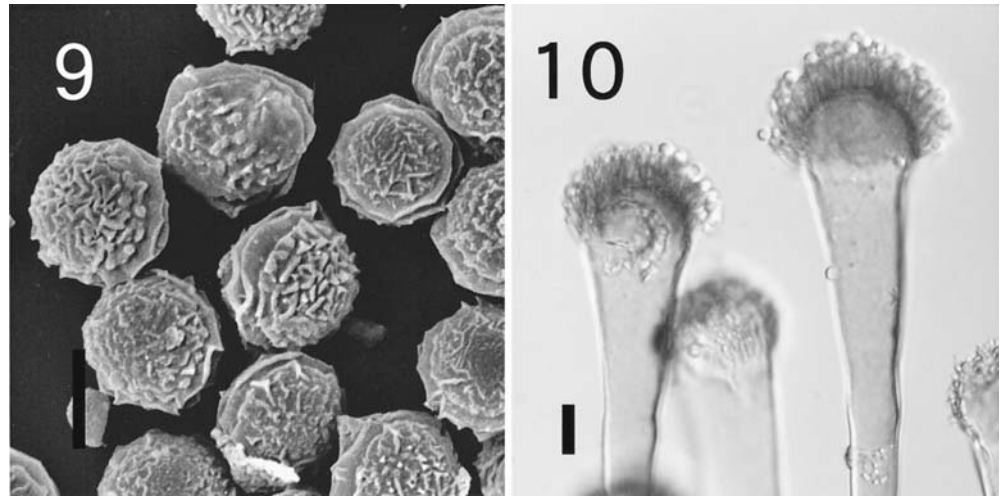
Figs. 3–8. 3–6 *Neosartorya indohii*. 3 Asci. 4 Ascospores. 5 Ascospores (SEM). 6 Aspergillum. 7,8 *Neosartorya tsurutae*. 7 Asci. 8 Ascospores. Bars 3, 4, 6–8 10 μm ; 5 5 μm



Colonies on MEA spreading broadly, attaining a diameter of 85 mm within 14 days at 25°C or within 7 days at 37°C, white to yellowish-grey (3D2) or greyish-green (1D3), with a thin, often submerged vegetative mycelium, granular in appearance due to the very abundant production of cleistothecia, loosely overgrown by aerial hyphae; conidiogenesis abundant; reverse yellowish-white to pale yellow.

Cleistothecia superficial, scattered, white to yellowish-white, globose to subglobose, 150–250 μm in diameter, surrounded by a loose covering of aerial hyphae; peridium hyaline to pale yellowish-brown, thin, membranaceous, consisting of angular cells, 4–13 μm in diameter. Asci 8-spored, globose to subglobose or ovoid, 9.5–12 \times 11.5–14.5 μm , evanescent at maturity. Ascospores hyaline to pale yellowish-brown, broadly lenticular, spore body 5.5–6.5 \times

Figs. 9,10. *Neosartorya tsurutae*.
9 Ascospores (SEM).
10 Aspergilla. Bars **9** 5µm;
10 10µm



4–5.5µm, provided with four equatorial crests that are up to 0.5–0.8µm wide with convex surfaces rugulose to ruminant (SEM).

Specimen examined: CBM-FA-933 (holotype), a dried culture of an isolate from soil in a tropical rain forest, Rio Urubu, President Figueiredo city, Amazonas st., Brazil (100km north from Manaus), collected and developed in the laboratory of Universidade Católica for Pernambuco, Recife, Pernambuco st., Brazil, by Y. Horie, Nov. 12, 2001 (isolate number 01-BA-739-2). Isotype: IFM 52114.

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads small, greyish-green, radiate to loosely columnar, 80–165 × 10–20µm. Conidiophores arising from aerial hyphae or the basal mycelium, hyaline to pale yellowish-brown, smooth, up to 735µm long, 6–10µm wide at the middle; vesicles hyaline, hemispherical to flask-shaped, 20–30µm in diameter. Aspergilla uniseriate; phialides hyaline to olivaceous-grey, 5–7 × 1.5–2.5µm, covering the upper half of the vesicle. Conidia hyaline to pale greyish-green, ovoid to broadly ellipsoidal, 2.5–3.5 × 2.5µm, smooth.

Colonies on CzA spreading broadly, attaining a diameter of 57–60mm in 14 days at 25°C, white-olive (2E3) or greyish-brown (5D3), consisting of a thin mycelial felt, zonate; cleistothecia few in number; conidiogenesis abundant; reverse yellowish-white (4A2) to greyish-yellow (4B3).

Colonies on OA spreading broadly, attaining a diameter of 80–85mm in 14 days at 25°C, orange-white (5A2), consisting of a thin mycelial felt, producing very abundant cleistothecia in a loose layer on the mycelial felt; conidiogenesis few in number; reverse yellowish-white (4A2).

The ascospores of this species are characterized by four equatorial crests and rugulose to ruminant convex surfaces. *Neosartorya quadricincta* also produces ascospores with four equatorial crests, but their convex surfaces are slightly reticulate or irregularly ridged.

Key to species of *Neosartorya*

1. Heterothallic 2
1. Homothallic 6
2. Ascomata persistently white in color 3
2. Ascomata pale yellow to light yellow 4
3. Ascospores with two equatorial crests and convex surfaces distinctly cerebriform; conidia globose to subglobose *N. fennelliae*
3. Ascospores with two broadly equatorial crests and convex surfaces echinulate; conidia ellipsoidal *N. nishimurae*
4. Ascospores with two equatorial crests 5
4. Ascospores with two equatorial or several irregular crests and convex surfaces tuberculate; conidiophores smooth *N. udagawae*
5. Ascospores convex surfaces almost smooth; conidiophores conspicuously roughened *N. spathulata*
5. Ascospores convex surfaces tuberculate or lobate-reticulate; conidiophores smooth *N. otanii*
6. Ascospores showing distinct equatorial crests 7
6. Ascospores without distinct equatorial crests 23
7. Ascomata and enveloping hyphae white to pale yellow or cream in color 8
7. Ascomata and enveloping hyphae yellow, golden, or orange in color 21
8. Ascospores showing entire equatorial crests 9
8. Ascospores showing serrate or incised equatorial crests *N. indohii*
9. Ascospores with four equatorial crests 10
9. Ascospores with two equatorial crests 11
10. Ascospore convex surfaces slightly reticulate or irregularly ridged *N. quadricincta*
10. Ascospore convex surfaces rugulose to ruminant *N. tsurutae*
11. Convex surfaces of ascospores reticulate 12
11. Convex surfaces of ascospores otherwise 15
12. Convex surfaces of ascospores obtusely and finely reticulate *N. hiratsukae*

12. Convex surfaces of ascospores distinctly and more largely reticulate 13
13. Convex surfaces of ascospores reticulate or somewhat irregularly reticulate *N. fischeri*
13. Convex surfaces of ascospores regularly microreticulate 14
14. Vesicles hemispherical to flask-shaped *N. tatenoi*
14. Vesicles ellipsoidal to nearly clavate *N. delicata*
15. Ascospores with closely appressed and very low equatorial crests: convex surfaces microtuberculate *N. sublevispora*
15. Ascospores with distinctly separated equatorial crests 16
16. Convex surfaces of ascospores tuberculata (SEM), nearly smooth (LM); equatorial crests widely separated *N. glabra*
16. Convex surfaces distinctly ornamented 17
17. Ascospores with less widely separated equatorial crests 18
17. Ascospores with widely separated equatorial crests 19
18. Convex surfaces of ascospores rough to distinctly spinulose *N. spinosa*
18. Convex surfaces of ascospores ornamented by roughly triangular flaps of tissue *N. pseudofischeri*
19. Convex surfaces of ascospores microaculeate and echinulate with spines or microverrucose 20
19. Convex surfaces of ascospores with roughly circularly arranged projections and microtuberculate *N. takakii*
20. Convex surfaces of ascospore microaculeate and echinulate with long spines *N. botucatensis*
20. Convex surfaces of ascospores irregularly roughened by microverrucose and small triangular projections *N. paulistensis*
21. Ascomata large (mostly more than 200µm in diameter); convex surfaces of ascospores echinulate *N. aureola*
21. Ascomata small, less than 200µm in diameter; convex surfaces verrucose 22
22. Ascospores with prominent equatorial crests; ascomata pure yellow; conidia with microverrucose wall *N. stramenia*
22. Ascospores with low equatorial crests; ascomata orange; conidia with labate-reticulate wall *N. aurata*
23. Ascospores with a very irregular ornamentation composed of several narrow ridges and triangular or verrucosa projections *N. primulina*
23. Ascospores ornamentation by several ridges, presenting ribbed or somewhat reticulate pattern *N. multiplicata*

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