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journal homepage: [www.elsevier.com/locate/myc](http://www.elsevier.com/locate/myc)**Short communication****Coprinopsis novorugosobispora sp. nov., an agaric ammonia fungus from Beijing, China**Toshimitsu Fukiharu<sup>a,\*</sup>, Kiminori Shimizu<sup>b</sup>, Ruoyu Li<sup>c</sup>, Jay Kant Raut<sup>d</sup>, Saori Yamakoshi<sup>e</sup>, Yoshikazu Horie<sup>b</sup>, Noriko Kinjo<sup>f</sup><sup>a</sup> Natural History Museum and Institute, Chiba, Aoba-cho 955-2, Chuo-ku, Chiba 260-8682, Japan<sup>b</sup> Medical Mycology Research Center, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8673, Japan<sup>c</sup> Research Center for Medical Mycology, Peking University First Hospital, No. 8 Xishiku Street, Xicheng District, Beijing 100034, China<sup>d</sup> Nepal Academy of Science & Technology, GPO Box 3323, Khumaltar, Lalitpur, Nepal<sup>e</sup> Ariake 2257-45, Hotaka, Azumino-shi, Nagano 399-8301, Japan<sup>f</sup> College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Kounodai 2-8-30, Ichikawa-shi, Chiba 272-0827, Japan

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## ABSTRACT

*Coprinopsis novorugosobispora* (Basidiomycota, Psathyrellaceae), a new ammonia fungus, fructificated from the soil sample collected in Beijing, China. In macro- and micro-morphology, this species is similar to *C. rugosobispora* reported from Belgium, but differs in having smaller basidiospore and rougher basidiospore ornamentation. This is the first report of ammonia fungi from continental China.

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The ammonia fungi (Sagara 1975, 1992) are known to form a fungal community that appears to be restricted to sites of animal materials such as decomposing carcasses and animal waste. This group of fungi is also stimulated to fruit by the addition of urea or aqueous ammonia or other related nitrogenous materials to forest soils (Sagara 1975). About 50 species have been recorded as ammonia fungi, but the species composition is different at each site (Sagara 1992). Among them, *Coprinopsis phlyctidospora* has been long time considered as a single species, but recent studies have revealed that *C. phlyctidospora* is actually a species complex composed of a number of different species (Fukiharu et al. 2011; Raut et al.

2011). In this study, we report a new ammonia fungus fructificated from a soil sample collected in Beijing, China.

Field soil was collected from 5 plots at two sites: site 1, Shidu, Fangshan District, Beijing, 200 m alt., *Pinus* dominated coppice wood, 2 plots, 15 August 1998; site 2, Fahai Si Temple, Shijingshan District, Beijing, 100 m alt., *Pinus* dominated coppice wood, 3 plots, 17 August 1998. From these sites, soil samples (mixtures of humus and litter layers) were collected. From these soil samples, ammonia fungi were isolated from the soil according to Sagara (1975). To stimulate fruiting of ammonia fungi, aqueous urea (granular fertilizer; 20 mg N/g dry weight soil) was applied to the collected soil/litter

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mixtures in a pot, and incubated at 15 or 23 °C under a 12 h light/12 h dark lighting regime for 30–120 d. Sterile water was applied every 3–7 d to avoid desiccation of the incubating soil mixture. Basidiomata observed were collected and mycelia were isolated from each basidioma. Cultures were grown at 25 °C under a 12 h light/12 h dark lighting regime on MYC medium consisting of 10 g Malt extract (Difco), 2 g Yeast extract (Difco) and 2 g Casamino-acid (Difco) per 1 L with 1.2% agar (Junsei Chemical Co., Ltd, Japan) for about 2–3 wk.

Anatomical observations and measurements were made on material mounted in 28% aqueous ammonia. Basidiospores were measured based on the following statistics: Q, quotient of spore length and spore width in face view;  $Q_m$ , the means of Q-values ( $\pm$ standard deviation). For scanning electron microscope (SEM) observation of basidiospore ornamentation, a small portion of a dried specimen was rehydrated in 28% aqueous ammonia, fixed in 5% osmium acid vapor, coated with platinum–palladium in an ion sputter-coater (Hitachi E-1030; Hitachi, Tokyo Japan), and observed under a SEM (Hitachi S-800) operating at 15.0 kV. All descriptions of macro- and microscopic characters were obtained from cultivated basidiomata. Color notation used in the species description is according to [Kornerup and Wanscher \(1978\)](#). Specimens examined are deposited in the Natural History Museum and Institute, Chiba (CBM).

*Coprinopsis novorugosobispora* Fukiharuru & Yamakoshi sp. nov.

Figs. 1, 2.

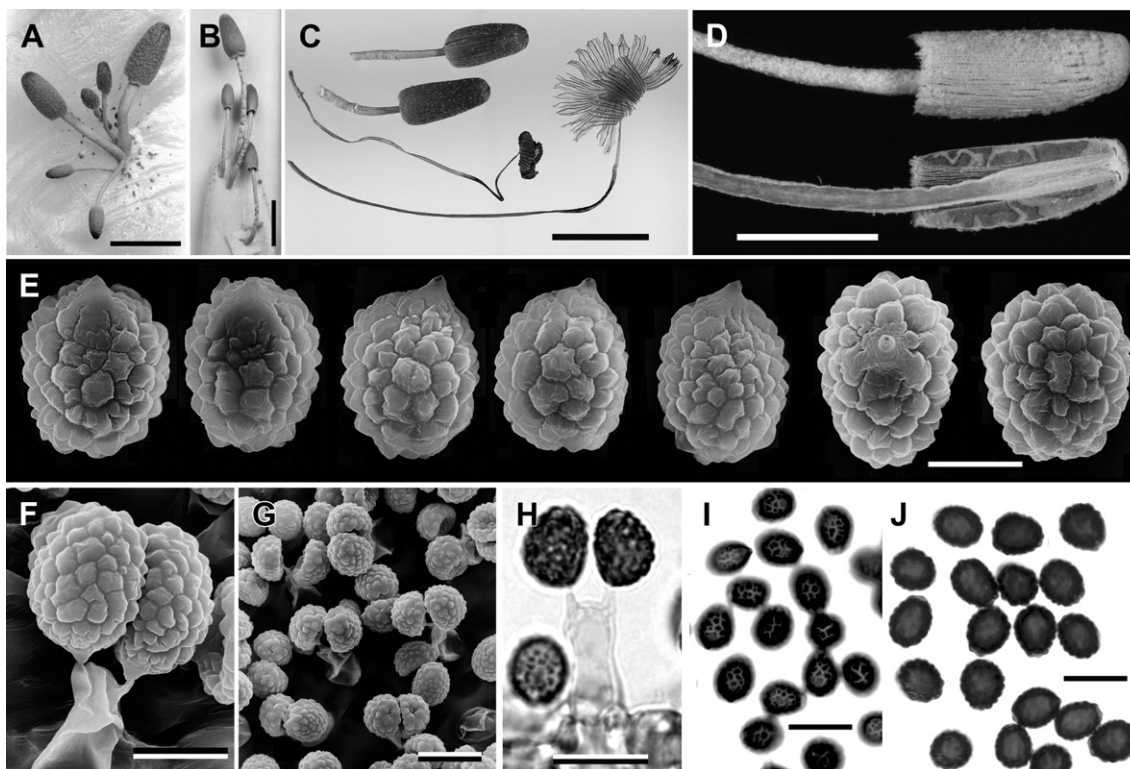
Mycobank no.: MB801285.

This species is characterized by having diverticulate veil elements, two-spored basidia and warty ornamented basidiospores and differs from the closest species, *Coprinopsis rugosobispora*, in having smaller basidiospores with more rough surface ornamentation.

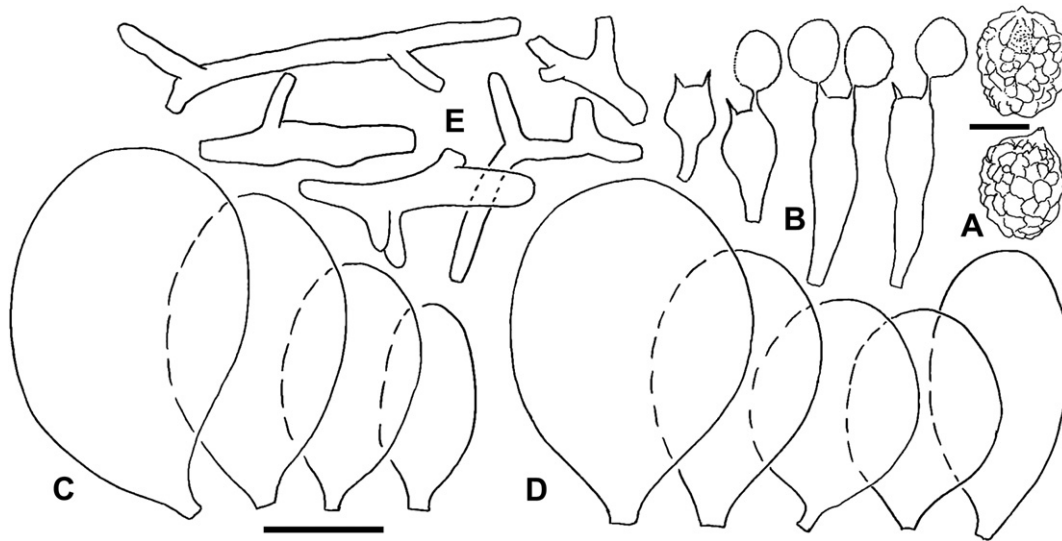
Holotype: China, coll. T. Fukiharuru, in Natural History Museum and Institute, Chiba (CBM-FB-21471).

**Eymology:** The Latin *novo-* referring to new species, and *-rugosobispora* reflects the morphological resemblance of this species to *C. rugosobispora*.

Pileus 3–7 mm broad, 5–12 mm high in button stage, when young ellipsoid to ovoid, later convex to plane, 15–20 mm broad when expanded, radially sulcate, at length the edge somewhat recurving and splitting irregularly, pileipellis color at first white to grayish orange (6B3), surface when young covered with white, woolly scales (Fig. 1A–D), sometimes with cortinate veil in very young stage (Fig. 1B, D), later almost glabrous or veil remaining only in the center. Flesh very thin, fragile, white, taste mild, odorless. Lamellae free, crowded (number of lamellae reaching stipe = 35–45), narrow (1 mm wide), edge slightly pruinose, at first white, then grayish, finally blackish, deliquescent. Stipe up to 40–70 mm  $\times$  0.6–2 mm, cylindrical, equal or somewhat tapering upward, sometimes the base clavate, not rooting, hollow, fragile, surface white, at first lanate (Fig. 1D), soon becoming smooth. Basidiospores black in mass, dark-brown (8F8, ornamentation area) and reddish-brown (8D7, cracked area) under the



**Fig. 1** – *Coprinopsis novorugosobispora*. **A**: Basidiocarps (CBM-FB-21398) **B**: Basidiocarps (CBM-FB-21798). **C**: Basidiocarps (CBM-FB-21471, holotype). **D**: Basidiocarps (CBM-FB-21469). Note: fruit bodies were photographed after cultivation on MYC medium at 25 °C for 8–12 days. **E–G**: Basidiospore ornamentations (CBM-FB-21396, SEM). **H–J**: Basidiospores (light microscopy). **H**: CBM-FB-21830. **I** & **J**: CBM-FB-21471 (Holotype). Note: **F–H** show two-spored basidia. Bars: **A–C**, 10 mm; **D**, 5 mm; **E** & **F**, 5 μm; **G–J**, 10 μm.



**Fig. 2** – *Coprinopsis novorugosobispora*. **A:** Basidiospores (face view, side view; CBM-FB-21396 and CBM-FB-21467). **B:** Basidia with basidiospores (CBM-FB-21469). **C:** Pleurocystidia (CBM-FB-21469). **D:** Cheilocystidia (CBM-FB-21469). **E:** Veil hyphae (CBM-FB-21469). Bars: A, 5  $\mu\text{m}$ . B–E, 20  $\mu\text{m}$ .

microscope, ovoid to ellipsoid, with warty ornamentation, with a clear plage (Fig. 1E, I),  $8.4\text{--}12.7 \times 6.5\text{--}9.8$  (face view)  $\times 5.5\text{--}9.2$  (side view)  $\mu\text{m}$  (dimensions including ornamentation): two types of basidiospore are recognized (Fig. 3); normal type is abundant (95–97%), dimensions including ornamentation:  $8.4\text{--}10.4$  ( $9.5 \pm 0.5$ : mean  $\pm$  SD,  $n = 40$ )  $\mu\text{m}$  long,  $6.7\text{--}8.6$  ( $7.4 \pm 0.4$ ,  $n = 20$ )  $\mu\text{m}$  broad in face view,  $6.2\text{--}7.9$  ( $7.3 \pm 0.5$ ,  $n = 20$ )  $\mu\text{m}$  in side view,  $Q = 1.1\text{--}1.4$ ,  $Q_m = 1.3 \pm 0.07$  ( $n = 20$ ), dimensions without ornamentation:  $7.2\text{--}9.1$  ( $8.5 \pm 0.4$ ,  $n = 40$ )  $\mu\text{m}$  long,  $6.2\text{--}7.5$  ( $6.8 \pm 0.3$ ,  $n = 20$ )  $\mu\text{m}$  broad in face view,  $5.5\text{--}7.2$  ( $6.6 \pm 0.5$ ,  $n = 20$ )  $\mu\text{m}$  in side view,  $Q = 1.1\text{--}1.4$ ,  $Q_m = 1.3 \pm 0.06$  ( $n = 20$ ), with a central germ pore  $0.9\text{--}1.2$   $\mu\text{m}$  wide; big size type is rare (5–3%): dimensions

including ornamentation,  $11.0\text{--}12.7$  ( $11.7 \pm 0.4$ : mean  $\pm$  SD,  $n = 40$ )  $\mu\text{m}$  long,  $8.6\text{--}9.8$  ( $9.2 \pm 0.4$ ,  $n = 20$ )  $\mu\text{m}$  broad in face view,  $7.9\text{--}9.2$  ( $8.5 \pm 0.6$ ,  $n = 20$ )  $\mu\text{m}$  in side view,  $Q = 1.2\text{--}1.4$ ,  $Q_m = 1.3 \pm 0.06$  ( $n = 20$ ), dimensions without ornamentation:  $9.9\text{--}11.5$  ( $10.7 \pm 0.4$ ,  $n = 40$ )  $\mu\text{m}$  long,  $7.9\text{--}8.9$  ( $8.3 \pm 0.4$ ,  $n = 20$ )  $\mu\text{m}$  broad in face view,  $7.2\text{--}7.9$  ( $7.4 \pm 0.3$ ,  $n = 20$ )  $\mu\text{m}$  in side view,  $Q = 1.2\text{--}1.4$ ,  $Q_m = 1.3 \pm 0.08$  ( $n = 20$ ), with a central germ pore  $1.0\text{--}1.2$   $\mu\text{m}$  wide. Basidia  $16\text{--}33 \times 6.5\text{--}8.0$  (long type:  $28\text{--}33 \times 6.8\text{--}7.5$ , short type:  $16\text{--}20 \times 7.5\text{--}8.0$ )  $\mu\text{m}$ , 2-spored (Figs. 1F–H, 2B). Pleurocystidia  $35\text{--}60 \times 15\text{--}38$   $\mu\text{m}$ , ellipsoid to obovoid, thin-walled, hyaline (Fig. 2C). Cheilocystidia  $26\text{--}58 \times 20\text{--}37$   $\mu\text{m}$ , subglobose to ellipsoid, numerous, thin-walled, hyaline (Fig. 2D). Veil on the pileal surface composed of thin-walled, diverticulate, hyaline hyphae,  $40\text{--}70 \times 4\text{--}10$   $\mu\text{m}$  (Fig. 2E). Clamp-connections present on cheilocystidia hyphae.

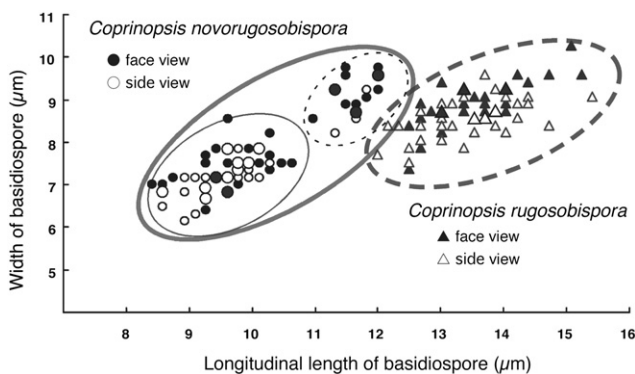
Specimens examined: all specimens in this study were cultivated from the stock culture, isolated from urea-treated soil of coppice woods, Beijing, China: CBM-FB-21471, Holotype; CBM-FB-21383, CBM-FB-21396, CBM-FB-21398, CBM-FB-21467, CBM-FB-21468, CBM-FB-21469, CBM-FB-21470, CBM-FB-21553, CBM-FB-21798, CBM-FB-21830, Paratypes.

Habit and habitat: this fungus was observed at a high frequency in each urea-treated soil pot, indicating that it is an ammonia fungus (Sagara 1975, 1992).

Distribution: Beijing, China.

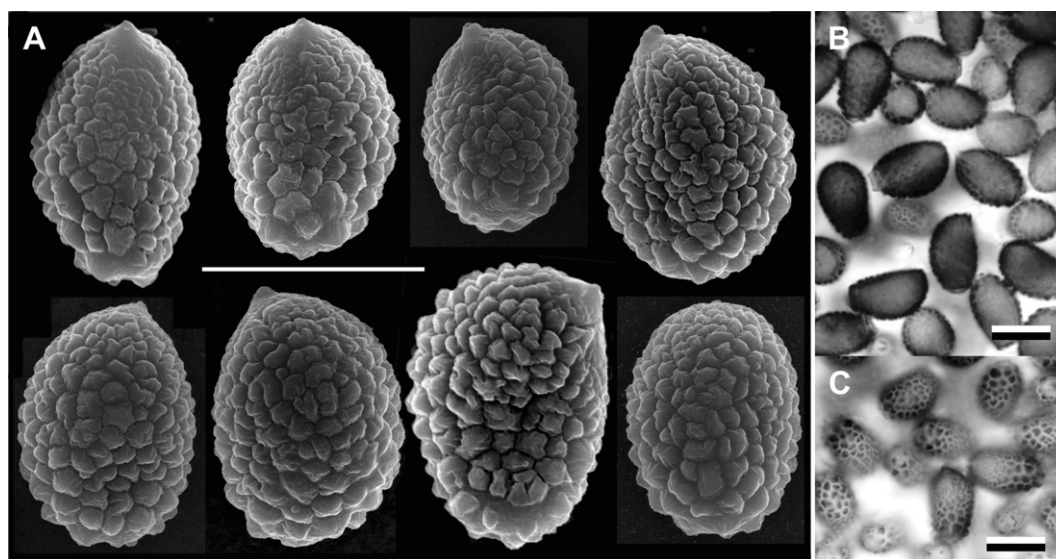
Other specimens examined: *Coprinopsis rugosobispora*: BR-44338-09 (Holotype, National Botanic Garden of Belgium, Geesink and Imler 1979).

Notes. According to the traditional *Coprinus* Pers. taxonomy, this species belongs to the genus *Coprinus*, Section *Coprinus* Singer, subsect. *Alachuani* Singer (Singer 1986) because of its diverticulate veil elements, i.e., the veil is composed of filamentous hyphae with small side-branches and branchlets. In this subsection, five species have been recognized to have warty ornamented basidiospores, i.e., *Coprinopsis*



**Fig. 3** – Size differences in basidiospores of *Coprinopsis novorugosobispora* and *C. rugosobispora*. Thick solid line: *C. novorugosobispora*, from spore print of cultured basidiomata, CBM-FB-21471 (Holotype), thin solid line: normal type ( $n = 57$ ), thin broken line: large size type ( $n = 18$ ), thick broken line ( $n = 66$ ): *C. rugosobispora*, from basidioma, BR-44338-09 (Holotype). Larger circles (*C. novorugosobispora*) and triangles (*C. rugosobispora*) indicate more than two basidiospores of identical sizes.





**Fig. 4** – *Coprinopsis rugosobispora* (BR-44338-09, holotype). **A:** Basidiospores (SEM). **B:** Basidiospores (light microscopy, focused at median plane). **C:** Basidiospores (light microscopy, focused at tangential plane) Bars: 10  $\mu$ m.

*austrophlyctidospora* Fukiharu, *C. echinospora* (Buller) Redhead, Vilgalys & Moncalvo, *C. neophlyctidospora* Raut, Fukiharu and A. Suzuki, *C. phlyctidospora* (Romagn.) Redhead, Vilgalys & Moncalvo, *C. rugosobispora* (J. Geesink & Imler) Redhead, Vilgalys & Moncalvo (Orton and Watling 1979; Moser 1983; Uljé 2005; Fukiharu et al. 2011; Raut et al. 2011).

*Coprinopsis rugosobispora* and *C. novorugosobispora* are distinguished from other species in having two-spored basidia (Fig. 1F–H). *Coprinopsis novorugosobispora* is separated from *C. rugosobispora* in having smaller basidiospores (Fig. 3) and also having a smaller spore value of  $Q$  ( $Q_m = 1.3$ ), i.e., *C. rugosobispora* basidiospore (holotype): dimensions including ornamentation, 12.0–15.4 ( $13.5 \pm 0.7$ : mean  $\pm$  SD,  $n = 40$ )  $\mu$ m long, 7.4–10.3 ( $8.9 \pm 0.5$ ,  $n = 20$ )  $\mu$ m broad in face view, 7.7–9.6 ( $8.5 \pm 0.5$ ,  $n = 20$ )  $\mu$ m in side view,  $Q = 1.4$ –1.7,  $Q_m = 1.5 \pm 0.07$   $\mu$ m ( $n = 20$ ); dimensions without ornamentation: 11.0–14.9 ( $12.7 \pm 0.8$ ,  $n = 40$ )  $\mu$ m long, 6.9–9.6 ( $8.1 \pm 0.6$ ,  $n = 20$ )  $\mu$ m broad in face view, 7.0–8.4 ( $7.9 \pm 0.4$ ,  $n = 20$ )  $\mu$ m in side view,  $Q = 1.4$ –1.7,  $Q_m = 1.6 \pm 0.07$  ( $n = 20$ ). Geesink and Imler (1979) also described the basidiospore size of *C. rugosobispora* as 12–13.75  $\times$  8–8.75  $\mu$ m (without ornamentation), and their description well agrees with our type study as demonstrated above. *Coprinopsis novorugosobispora* is also distinguished from *C. rugosobispora* in having more roughly ornamented basidiospores (see Figs. 1E and 4A). Phylogenetic analysis with molecular data for the *C. phlyctidospora* species complex including *C. novorugosobispora* is underway and will be presented elsewhere.

Interestingly, this new species forms two kinds of basidiospores of different sizes (Fig. 3). All basidia seemed to form these two kinds of spores, though it may be considered that the presence of significantly larger basidiospores is the sign of the existence of one-spored basidia. It is also worth noting that spores of significantly different sizes were obtained from a single fruiting body. Uljé (2005) also reported “*C. rugosobispora*” auct. non (J. Geesink & Imler) Redhead, Vilgalys & Moncalvo from Netherlands but this species is considered to be

*C. novorugosobispora* based on the reported basidiospore size. We have not yet collected any basidioma of *C. novorugosobispora* from the field. However, since *C. austrophlyctidospora*, a member of the *C. phlyctidospora* species complex (Fukiharu et al. 2011), indicated the same morphological characters in both field and cultivated specimens, *C. novorugosobispora* described in this paper can be considered to be morphologically similar to its field specimens.

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