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Coprinopsis novorugosobispora (Basidiomycota, Agricales), an ammonia fungus new to Canada

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Abstract

Fruiting of *Coprinopsis novorugosobispora* was effectively stimulated by urea treatment in the soil collected from aspen forest in Canada. A complete description and illustration on the fungus with its phylogenetic analysis based on nuclear rRNA gene sequences in ITS regions are provided. The report represents a new record in American continent.

Key words – Aspen – Coprinoid – saprobic – species complex – urea

Introduction

Ammonia fungi are defined as a chemoecological group of fungi that sequential occurrence is stimulated on natural soils by treatment of aqua ammonia or ammonia-releasing nitrogenous materials (Sagara 1975). To date, the study of ammonia fungi has been done in diverse geographical areas following application of urea in the field and/or laboratory such as in Australia (Suzuki et al. 1998, 2002a, 2003, Nagao et al. 2003, Fukiharu et al. 2011), Canada (Suzuki 2006, Raut et al. 2011), China (Fukiharu et al. 2012), Japan (Sagara and Hamada 1965, Sagara 1975, 1992, Suzuki 1992, Fukiharu and Hongo 1995, Yamanaka 1995a–c, Fukiharu & Horigome 1996, Fukiharu et al. 1997, 2014, Sato & Suzuki 1997, Suzuki et al. 1998, 2002b, 2003, Imamura 2001, He & Suzuki 2004, Imamura & Yumoto 2004, 2008, Sagara et al. 2008), New Zealand (Suzuki et al. 2002a, 2003, Fukiharu et al. 2011), Taiwan (Wang & Sagara 1997), Thailand (Manusweeraporn et al. 2013), UK (Sagara et al. 2008), US (Sagara 1992) and Vietnam (Ho et al. 2014)) and above 70 species of ammonia fungi have been recorded. However, ammonia fungi in boreal region have not yet been well investigated. We, therefore, surveyed ammonia fungi in boreal region near Edmonton, Canada by artificial urea application for the first time and collected a new taxon *Coprinopsis neophlyctidospora* Raut, Fukiharu & A. Suzuki (Raut et al. 2011). This is the second report of the ammonia fungi in Basidiomycota from Canada. At the beginning it was reported as *C. rugosobispora* (J. Geesink & Imler) Redhead, Vilgalys & Moncalvo (Raut et al. 2010) but later based on the description of *C.*

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novorugosobispora Fukiharu & Yamakoshi from China (Fukiharu et al. 2012) and detailed phylogenetic study with type specimens of former and latter both here it is described as *C. novorugosobispora*.

Materials & Methods

Isolates and morphology

Basidiomata were obtained from the isolate incubated at 25°C on malt yeast agar medium (malt extract 10 g/l (Difco, USA), yeast extract 2 g/l (Difco, USA) and agar 15 g/l (Nacalai Tesque, Japan) under lighting regime (12 h light/12 h dark). The isolate was obtained from a basidiomata collected on 8 July 2001 from the mixture of litter and soil collected from aspen forest (*Populus tremuloides* Michx.) followed by an application of urea (granular fertilizer; 46% nitrogen; 10 mg-N/g dry soil) after 38 days of incubation at 25°C under lighting regime (12 h light/12 h dark) associating with watering about 2 days interval.

All descriptions of macro- and microscopic features were obtained from cultivated basidiomata. Anatomical observations and measurements were made on material mounted in 25% aqueous ammonia. Microscopic terminology of basidiospores and cystidia were followed on Vellinga (1988). Color terms and notations used in this description are based on Kornerup and Wanscher (1978). Herbarium abbreviations are according to Holmgren and Holmgren (1998). Basidiospore statistics: x_m, the arithmetic mean of the spore length by spore width (± standard deviation) ; n, number of spores measured; Q, the quotient of spore length and spore width; Q_m, the mean of Q-values (± standard deviation). For scanning electron microscopes (SEM) observation of basidiospore, samples were rehydrated in 25% aqueous ammonia and fixed in 2.5% osmium acid, coated with platinum-palladium sputter in an ion sputter-coater (Hitachi E-1030; Hitachi, Japan), and observed under a SEM (Hitachi S-800; Hitachi, Japan) operating at 15.0 kV.

PCR Amplification and sequencing of ITS Region

Fungal strains were grown in a MY (malt yeast extract) liquid medium. Mycelia were harvested, squeezed with a paper towel, frozen and lyophilized. The dried mycelia were then ground with a spatula and re-suspended in a TES buffer [50 mM Tris-HCl (pH 7.5), 20 mM EDTA, 1% SDS], and soluble fractions were recovered by the centrifugation. The DNA was purified by a TE buffer [10 mM Tris(HCl, pH 8.0)1 mM EDTA] saturated phenol/chloroform/isoamyl alcohol (Nippon gene, Japan) extraction followed by an iso-propyl alcohol precipitation. After desiccation of the DNA pellet, the DNA was dissolved in a 30 µl TE buffer. For some samples, the genomic DNA was further purified by using a NucleoSpin Extract II (Macherey-Nagel, Germany) following the manufacture's recommendation. Primers ITS1 (5′-TCCGTAGGTGAACCTGTCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGTATGC-3′) were used to amplify the ITS1-5.8S-ITS4 ribosomal DNA region (full ITS region). PCR reactions were carried out using the Ex Taq (TakaraBio, Japan) according to the manufacture's protocol. PCR products were purified by using the NucleoSpin Extract II, and the DNA fragments were directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol. The reactions were then cleaned up using the Centri Sep (Princeton Separations) before analysis by the capillary electrophoresis on a 3130x DNA Analyzer (Applied Biosystems, USA). Sequences were assembled and edited using the ATSQ software (Genetyx, Japan). All nucleotide sequences were deposited in GenBank. The data set was aligned using Clustal X ver. 1.81 (Jeannmougin et al. 1998), and the resulting alignment was manually refined. The alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession number http://purl.org/phylo/treebase/phylows/study/TB2:S16166. A phylogenetic tree was constructed based on the neighbor-joining (NJ) method (Saitou & Nei 1987) using the NJ plot program (Perrière & Gouy 1996). The robustness of inferred NJ topologies was tested by the bootstrap value (Felsenstein 1985) with 1000 replicates. *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo was used as the out group. Sequence data used from the GenBank are listed in the Table 1 with their accession numbers.

Table 1 Taxa sampled for the phylogenetic analysis

Taxa	Isolate No.	Voucher specimen No.	Locality	GenBank Accession No.
Sequences generated in this study				
Coprinopsis novorugosobispora	SL[a]503001	CBM[d]-FB38004	Alberta, Canada	AB564411
		CBM-FB21471	Beijing, China	AB978534
C. rugosobispora		Br[e]-44338-09	Belgium	AB983245
Sequences retrieved from GenBank				
C. phlyctidospora (Romagn.) Redhead, Vilgalys & Moncalvo	NBRC[b]30478		Kyoto, Japan	AB071615
		CBM-FB24542	Kochi, Japan	AB071613
		1026[f](Uljé)		AB071608
C. neophlyctidospora	SL503201	CBM-FB33899	Alberta, Canada	AB564407
	SL503202	CBM-FB33901	Alberta, Canada	AB564406
	SL503203	CBM-FB33894	Alberta, Canada	AB564408
C. austrophlyctidospora Fukiharu	SL503401	CBM-FB29564	North Island, NZ	AB071793
	SL503402	CBM-FB30247	North Island, NZ	AB071795
	SL503403	CBM-FB24556	W. Australia	AB071796
		E5808[g] (CSIRO)	W. Australia	AB071791
C. asiaticiphlyctidospora Fukiharu & Horigome		CBMb-FB-38668	Amami, Japan	AB818904
		CBM-FB-37217	Amami, Japan	AB817730
		CBM-FB-37218	Amami, Japan	AB817731
		CBM-FB-37220	Amami, Japan	AB817732
C. echinospora (Buller) Redhead, Vilgalys & Moncalvo		CBM-FB21629	Aomori, Japan	AB071798
		CBM-FB21725	Aomori, Japan	AB071800
		537[f] (Uljé)		AB071802
C. atramentaria	KACC[c]49358			AF345814

[a] SL numbers are stock cultures of Forestry and Forest Products Research Institute, Japan
[b]NBRC: Culture collection of NITE Biological Resource Center, Japan
[c]KACC: Culture collection of Korean Agricultural Culture Collection, Republic of Korea
[d]CBM: Specimen collection of Natural History Museum and Institute, Chiba, Japan
[e]Br : Voucher Specimen stored in National Botanic Garden of Belgium
[f]Voucher specimen stored by Dr. C.B. Uljé
[g]Voucher specimen deposited in the Herbarium of CSIRO, Perth, Australia

Results

Coprinopsis novorugosobispora Fukiharu & Yamakoshi in Mycoscience 54 (3): 226–230.

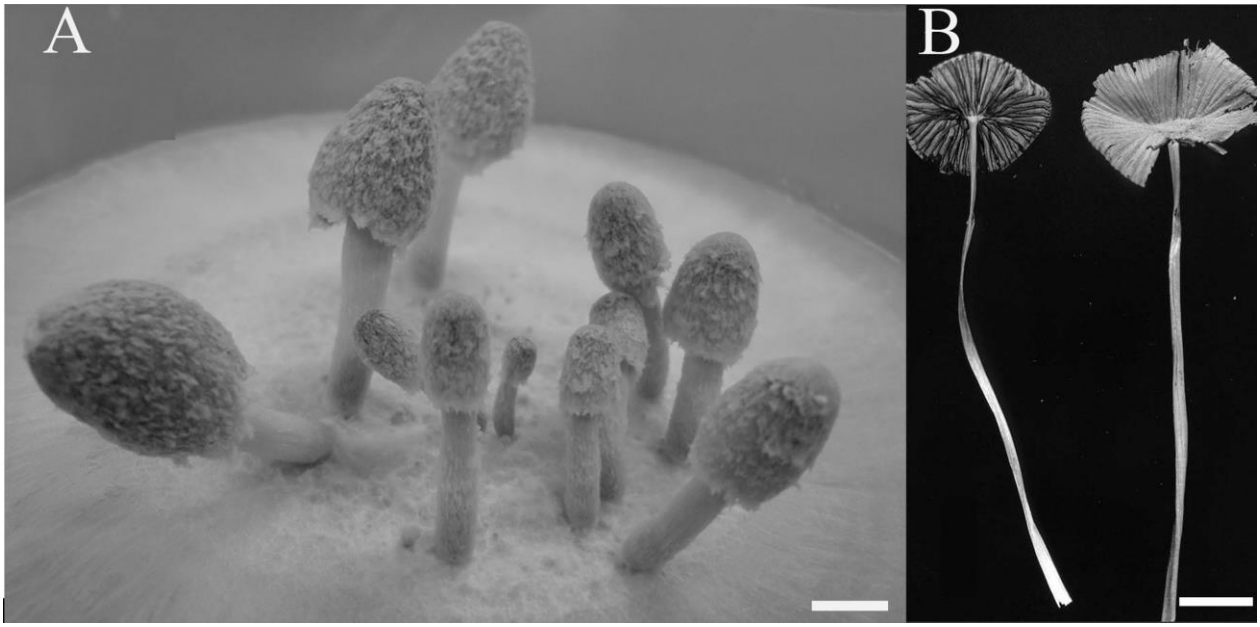

Fig. 1 – Macromorphology of *Coprinopsis novorugosobispora* collected from Canada. A–B Different developmental stages of basidiomata. Bars A–B = 5 mm.

Description based on Canadian specimens

Pileus 4–15 mm broad, 3–10 mm when still closed, up to 25 mm when expanded, ellipsoid or ovoid when young, later convex to plane, finally uplifted. Pileipellis color at first white soon becoming gray, surface when young densely covered with white radially arranged hairy-fibrillose scales, later almost glabrous or veil remaining only in the center, flesh very thin, fragile. Lamellae free, close to crowded (number of lamellae 25–50), with 0–3 lamellulae between two lamellae, deliquescent, first white, then gray, finally blackish. Stipe 40–90 × 1–3 mm, central, cylindrical, not rooting, fistulose, fragile, base slightly clavate, surface white at first with fibrillose scales soon becoming smooth (Fig. 1A–B). Basidiospores black in mass, dark red-brown under microscope, 9.8–11.7 × 8.3–9.6 µm (xm = 10.7 ± 0.4 × 9.0± 0.4 µm, Q = 1.1–1.3, Qm = 1.2 ± 0.1, n = 40), ovoid to ellipsoid with warty ornamentation, a central germ pore 1.6–2.3 (2.0 ± 0.3, n= 10) µm wide, a clear plage (Fig. 3). Basidia 20–30 × 7–8 µm, one to two spored (Fig. 2A). Pleurocystidia 55–65 × 25–35 µm, ellipsoid, utriform or broadly cylindrical, thin walled, hyaline (Fig. 2B–C). Cheilocystidia 35–55 × 20–30 µm, sub-globose, ellipsoid, narrowly ovoid, utriform or broadly cylindrical (Fig. 2D). Elements of veil on the pileal surface composed of thin-walled, diverticulate, hyaline hyphae, 40–90 × 5–7 µm (Fig. 2E). Clamp-connections observed on vegetative hyphae.

Habit & Habitat – Saprobic, solitary to gregarious, growing after application of urea (10 mg-N/g dry soil) in the soil collected from aspen (*Populus tremuloides*) forest in Canada.

Known Distribution – China, Canada

Specimens examined: Canada, Alberta, Chipman, Alberta (668 m, 53°34′N 113°31′W), collected from the isolate incubated at 25°C on MY agar medium under lighting regime (12 h light/12h dark). The isolate was obtained from a basidiomata occurred on urea amended forest soil of aspen (*P. tremuloides*) A. Suzuki (CBM-FB 38004, CBM-FB 38005).

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

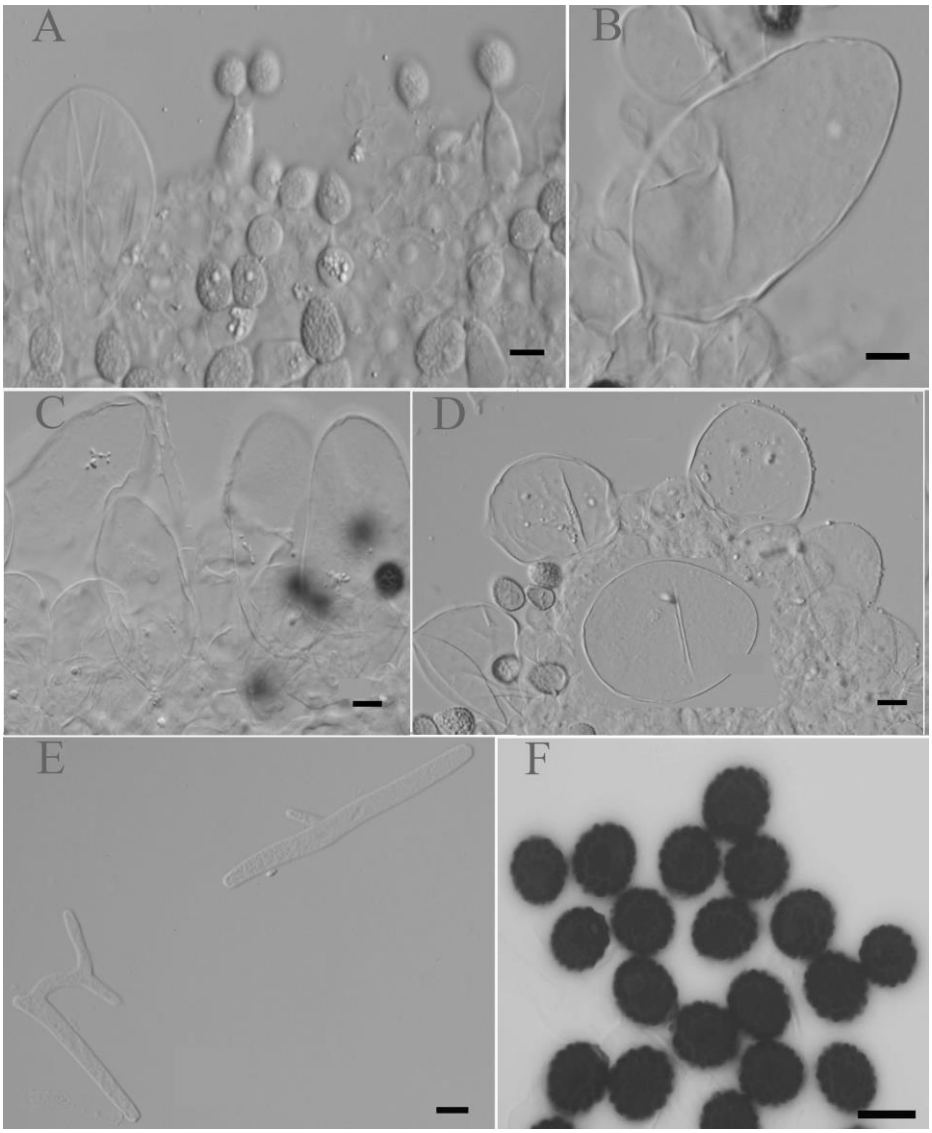

Fig. 2 – Micromorphology of *Coprinopsis novorugosobispora* collected from Canada. A: Basidia with basidiospores B–C: Pleurocystidia D: Cheilocystidia E: Veil elements F: basidiospores (under light microscope) Bars: A–F = 20 μm.

Discussion

The macro- and microscopic features of the Canadian specimen described above (Figs. 1–3), agree well with the original description of *C. novorugosobispora* from China (Fukiharu et al. 2012). Phylogenetic analysis also indicated that the samples from China and from Canada are placed in the same clade (Fig. 4). *Coprinopsis novorugosobispora* was phylogenetically clearly apart from *C. rugosobispora* with the high bootstrap value, but both species located in the group of *C. phlyctidospora* complex (Raut et al. 2011, Fukiharu et al. 2014). However, *C. rugosobispora* and *C. phlyctidospora* were not very well separated from each other (Fig. 4). Thus, more specimens should be determined to discuss whether *C. rugosobispora* (Geesink and Imler 1979) is an independent species or just a morphological variety of *C. phlyctidospora*. There have been a few records for the ammonia fungi (*Amblyosporium botrytis* Fresen., *Ascobolus denudatus* Fr., and *Coprinopsis neophlyctidospora*) in boreal forest of the North America (Suzuki 2006, Raut et al. 2011), and this report is the second one of *Coprinopsis* species from the boreal forest. In the preliminary study of ammonia fungi in the same forest, we have detected more saprobic species such as Coprinoid and *Panaeolus* spp., some ectomycorrhizal species such as *Hebeloma* spp. More study is necessary to reveal the complete community structure of the ammonia fungi in boreal forest in North America.

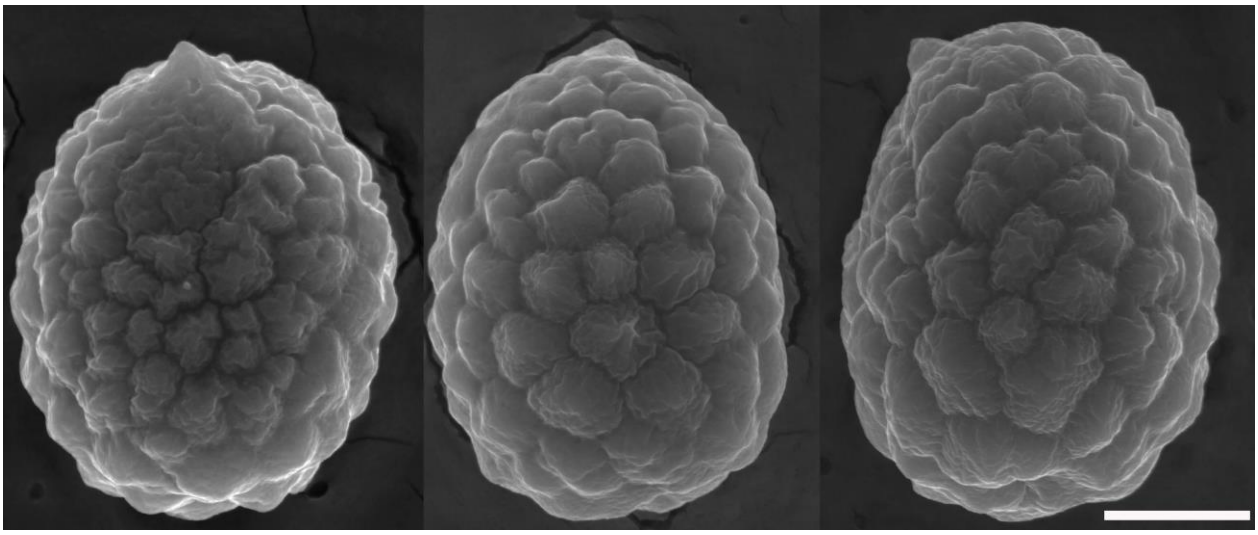

Fig. 3 – Basidiospores of *Coprinopsis novorugosobispora* collected from Canada. Face view, side view, back view from the left to right. Bar = 3 μm.

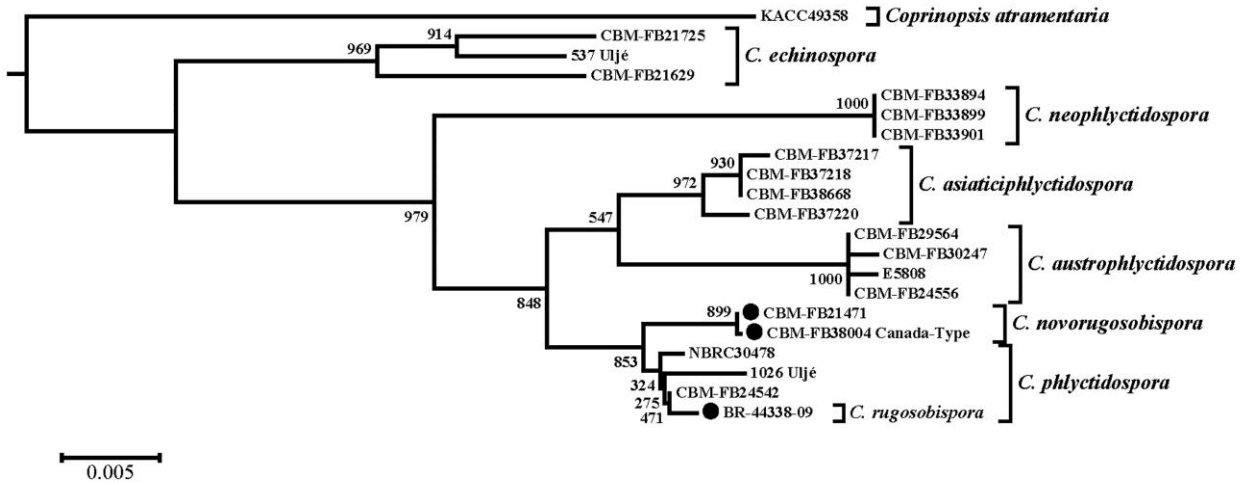

Fig. 4 – Phylogenetic tree constructed by the neighbor-joining (NJ) method (Saitou and Nei 1987) based on sequences of nuclear rRNA gene in ITS region. Numbers on the branches represent bootstrap values obtained from 1000 replication. The distance corresponding to five base changes per 1000 nucleotide position is indicated by the bar. *Coprinopsis atramentaria* was used as the outgroup taxon. Sidebar represents the inferred clades of *Coprinopsis* species. ● Sequences generated in this study.

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