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ITS rDNA variation of the *Coprinopsis phlyctidospora* (syn.: *Coprinus phlyctidosporus*) complex in the Northern and Southern Hemispheres

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Abstract *Coprinopsis phlyctidospora* (syn: *Coprinus phlyctidosporus*) from the Netherlands, Japan, New Zealand, and Australia can be segregated into two groups, northern and southern, based on the nucleotide sequences of their ITS regions. The mating type of a *C. phlyctidospora* isolate was tetrapolar. Mating reactions were compatible between monokaryotic testers derived from basidiospores of a Japanese isolate and dikaryotic isolates obtained from a wide geographic area in Japan. In contrast, mating between the Japanese monokaryotic and dikaryotic isolates from Australia and New Zealand were incompatible. These results indicated that *C. phlyctidospora* was complex and individuals currently recognized as *C. phlyctidospora* in the Northern Hemisphere and those in the Southern Hemisphere are distinct taxa. The relationship between the clades and the biogeography of the *C. phlyctidospora* complex are also discussed.

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Introduction

Coprinopsis phlyctidospora (Romagn.) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus phlyctidosporus* Romagn.) is found widely in the temperate regions of the Northern Hemisphere, e.g., France (Romagnesi 1945), Japan (Aoki and Hongo 1965), Italy (Leone 1978/1979a), England (Orton and Watling 1979), and the Netherlands (Uljé and Noordeloos 1997), but it is uncommon. We have found it occurs abundantly from Hokkaido Island (northeastern part of Japan; cool temperate region) to Iriomote Island (southwestern part of Japan; subtropical region) following application of a large amount of urea to the soil (Sagara 1975; Suzuki 1992; Fukiharu and Hongo 1995; Fukiharu and Horigome 1996; Fukiharu et al. 1997; Sato and Suzuki 1997; Suzuki and Toyokawa 1998/1999; He and Suzuki 2000). We have also collected this fungus in urea-treated plots in the North Island of New Zealand and near Perth in Western Australia (*cf.* Table 1). Morphological characters for all collections to date have broadly fitted within the species concept of *Coprinopsis phlyctidospora* (details unpublished). Thus, this fungus is considered to be distributed widely in the temperate and subtropical zones.

Species concepts and biogeographic speciation of widely distributed basidiomycetes have been investigated by methods based on morphology, mating compatibilities, and molecular genetics and phylogenetic analysis. Phylogenetic studies and their implications for understanding the biogeography of those saprobic or pathogenic basidiomycetes have been progressing significantly in recent years due to advancements in molecular techniques. For example, the shiitake mushroom comprises three morphological species: *Lentinula edodes* (Berk.) Pegler (continental and northeast Asia), *L. lateritia* (Berk.) Pegler (tropical Asia and Australia), and *L. novaezelandiae* (Stev.) Pegler (New Zealand) (Pegler 1983). Shimomura et al. (1992) found that

Table 1. List of specimens and isolates used in this study

Taxa	Voucher specimen no.	Isolate no.[a]	Locality	Dominating vegetation	Treatment[b]	Acc. no.[c]
Coprinopsis phlyctidospora (Romagn.) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus phlyctidosporus* Romagn.)	1026(Uljé)[d]		Prov. Zuid-Holland, The Netherlands			AB071608
	(CBM-FB24541)[e]	CHU3018	Aya, Miyazaki, Japan	*Quercus gilva, Quercus myrsinaefolia, Quercus glauca, Castanopsis cuspidata*	L	AB071617
	(CBM-FB24542)	CHU3004	Kubokawa-cho, Kochi, Japan	*Castanopsis cuspidata*	L	AB071613
		IFO30478	Iwakura, Kyoto, Japan	*Castanopsis cuspidata*	F	AB071615
		CHU01	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU02	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU03	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU04	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU05	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU06	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU07	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU08	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU09	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU10	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU11	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
		CHU12	Monokaryotic offspring of *C. phlyctidospora* IFO30478			
	(CBM-FB24539)	CHU3003	Kannami, Shizuoka, Japan	*Quercus acuta*	L	AB071609
	CBM-FB21061		Chiyoda-ku, Tokyo, Japan	*Quercus acuta*	L	AB071610
	(CBM-FB24544)	CHU3010	Kiyosumi, Chiba, Japan, site A[f]	*Quercus acuta, Castanopsis cuspidata*	F	AB071614
	(CBM-FB24548)	CHU3017	Kiyosumi, Chiba, Japan, site B	*Quercus acuta, Castanopsis cuspidata*	F	AB071616
	CBM-FB21611		Tsuta-onsen Aomori, Japan	*Fagus crenata*	L	AB071612
	CBM-FB21220		Kuromatsunai, Hokkaido, Japan	*Fagus crenata*	L	AB071611
	(CBM-FB24558)	CHU3002	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site C[g]	*Nothofagus menziesii, Nothofagus fusca*	F	AB071788

	(CBM-FB21222)	CHU3007	Riverhead, North Island, New Zealand	*Pinus radiata* (plantation)	F	AB071789
	(CBM-FB24568)	CHU3009	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site D	*Nothofagus menziesii*, *Nothofagus fusca*	F	AB071790
	(CBM-FB24562)	CHU3013	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site E[h]	*Nothofagus menziesii*, *Nothofagus fusca*	L	AB071792
	(CBM-FB29564)	CHU3014	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site E	*Nothofagus menziesii*, *Nothofagus fusca*	L	AB071793
	(CBM-FB29560)	CHU3015	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site E	*Nothofagus menziesii*, *Nothofagus fusca*	L	AB071794
	(CBM-FB30247)	CHU3016	Kaimanara State Forest Park, Taupo, North Island, New Zealand, site E	*Nothofagus menziesii*, *Nothofagus fusca*	L	AB071795
	(CBM-FB24556)	CHU3026	Dwellingup, near Perth, Western Australia, Australia, Site F[i]	*Eucalyptus marginata*, *Eucalyptus calophylla*	F	AB071796
	E5808 (CSIRO)[j]		Dwellingup, near Perth, Western Australia, Australia, Site G	*Eucalyptus marginata*, *Eucalyptus calophylla*	F	AB071791
Coprinopsis echinospora (Buller) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus echinosporus* Buller)	537 (Uljé)		Prov.Zuid-Holland, the Netherlands			AB071802
	CBM-FB21264		Kuromatsunai, Hokkaido, Japan	*Fagus crenata*	L	AB071798
	CBM-FB21629		Sukayu, Aomori, Japan	*Fagus crenata*	L	AB071799
	CBM-FB21725		Tsuta-onsen, Aomori, Japan	*Fagus crenata*	L	AB071800
	CBM-FB21733		Kurokawa, Yamato-cho, Miyagi, Japan	*Fagus crenata*	L	AB071801
		IFO30631	Iwakura, Kyoto, Japan	*Castanopsis cuspidata*	F	AB071803
Coprinopsis strossmayeri (Schulzer) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus rhizophorus* Kawam. ex Hongo & K. Yokoyama)		IFO30197	Kozuhara, Ibuki-cho, Shiga, Japan	*Cryptomeria japonica* (plantation)		AB071797

[a] Isolates CHU01-CHU12 are monokaryotic; other isolates are dikaryotic; isolates indicated by CHU numbers are stock cultures of Faculty of Education, Chiba University, Japan
[b] F, fruit-bodies obtained by urea treatment in field experiment; L, fruit-bodies obtained by urea treatment in laboratory experiment
[c] Sequence data deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases; Acc. no., accession number
[d] Dry specimen stored by Dr. C.B. Uljé
[e] Accession code in parentheses indicates dry specimens of the fruit-bodies obtained from the isolate shown in "Isolate no." DNA extraction was done by the isolate not by the dry specimens
[f] Site A is *ca.* 0.8 km from site B
[g] Sites C and E are *ca.* 25 km from site D; site C is *ca.* 10 m from site E
[h] In site E, a mixture of soils of L-, F-, and H-horizons, and upper layer of A-horizon, were collected from nondisturbed area
[i] Site F is *ca.* 5 m from site G
[j] Dry specimen deposited in the Herbarium of CSIRO, Perth, Australia

all three morphological species were interfertile and thus they regarded all three as a single species, *L. edodes*. Hibbett et al. (1998) recognized five independent lineages of *L. edodes sensu auct.* in Shimomura et al. (1992) based on rDNA sequences and inferred that it has a complex biogeographic history. *Armillaria mellea* (Vahl.: Fr.) P. Kumm. is another famous ubiquitous species. This fungus was once thought to be a morphologically variable species with a wide distribution and a very broad host range (Singer 1956). An indirect assessment of sexual incompatibility in the genus *Armillaria* has led to identification of biological species within *A. mellea sensu lato* (Korhonen 1978; Anderson and Ullrich 1979). The geographic differentiation of *A. mellea sensu stricto* was inferred from interfertility (Anderson et al. 1980) and similarity in basidiocarp morphology (Mottta and Korhonen 1980). Thereafter, high divergency of allopatric isolates (European group, western North American group, Asian group, eastern North American group) of *A. mellea sensu stricto* was inferred from phylogenetic analysis based on both the intergenic spacer region (IGS) and internal transcribed spacer region (ITS) regions of rDNA sequences (Coetzee et al. 2000). These results indicate that not only molecular phylogenetic study but also morphological studies and mating compatibility studies are necessary to reveal the phylogenetic and biogeographic diversity and species concepts of widely distributed fungus species.

Concerning *C. phlyctidospora*, little is known about its biogeographic variation in spite of records from extremely different geographic areas in both the Northern and Southern Hemispheres, as already mentioned. We therefore undertook to examine the genetic variation of *C. phlyctidospora* in relation to biogeography based on the nucleotide sequences of the ITS region of nuclear rDNA and inter- and intracompatibility of the isolates from Japan and those of New Zealand and Australia.

Materials and methods

Fungal specimens and cultures

Five dried basidiomata and 14 dikaryotic isolates of *Coprinopsis phlyctidospora*, five dried basidiomata and 1 dikaryotic isolate of *Coprinopsis echinospora* (Buller) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus echinosporus* Buller), and 1 dikaryotic isolate of *Coprinopsis strossmayeri* (Schulzer) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus rhizophorus* Kawam. ex Hongo & K. Yokoyama) were examined (see Table 1). *Coprinopsis echinospora* and *C. strossmayeri* were used as outgroups.

DNA preparation

A minute slice of dried pileus with basidiospores (10–20 mg) or a piece of dried dikaryotic mycelium (10–20 mg) was suspended in 500 µl extraction buffer [50 mM Tris-HCl pH 8.0, 125 mM ethylene diamine tetraacetic acid (EDTA), 100 mM NaCl, 2% (w/v) sodium *N*-lauroylsarcosinate, 1% (v/v) 2-mercaptoethanol]. DNA was extracted by the method of Nakada et al. (1994).

PCR amplification and ITS DNA sequencing

Primers used in polymerase chain reaction (PCR) amplification were ITS4 and ITS5 (White et al. 1990). The 50 µl reaction mixture contained 10 pmol each primer, about 10 ng template DNA, 250 µM each deoxynucleotide triphosphate (dNTP), 1.25 U KOD dash DNA polymerase (Toyobo, Osaka, Japan), and 5 µl 10× KOD dash buffer. The thermal cycler (TP3000; Takara, Kyoto, Japan) was programmed as follows: initial denaturation, 2 min at 95°C; then 30 cycles of 30 s at 95°C, 2 s at 52°C, 30 s at 74°C; and a final extension at 72°C for 5 min. After electrophoresis in 1% low me1ting point agarose gel (Sea Plaque GTG Agarose; FMC, Rockland, ME, USA), the amplified products were excised from the gel, according to standard protocols for DNA handling (Sambrook et al. 1989). The DNA fragments were cloned into pZErO™-2 (Invitrogen, Carlsbad, CA, USA). To avoid artifact DNA sequences caused by errors in DNA polymerization, at least three recombinants were selected from a batch of transformants and the homogeneity of all or the majority of DNA sequences was confirmed. DNA was sequenced by the dideoxy chain termination method (Sanger et al. 1977) with a SequiThermo EXCEL™ II DNA sequencing kit (Epicenter Technology, Madison, WI, USA), according to the manufacturer's recommendations. The sequencing primers used were fluorescent dye- (Cy5-) labeled M 13–20 and M 13-RV (Pharmacia Biotech, Uppsala, Sweden). Samples were separated by electrophoresis on an ALFred DNA sequencer (Pharmacia Biotech).

Phylogenetic analysis

Sequences were aligned using the CLUSTAL W multiple alignment program version 1.8 (Thompson et al. 1994). The aligned sequences were analyzed by the neighbor-joining method (Saitou and Nei 1987), using NEIGHBOR in PHYLIP version 3.5c package (Felsenstein 1993). The distance matrix was calculated using DNADIST with Kimura's two-parameter method, and the topology was tested with 1000 bootstrap trials.

Monokaryotic isolates

A dikaryotic isolate, *C. phlyctidospora* IFO30478, was inoculated on MY agar slant (malt extract; Difco, Detroit, MI, USA), 10 g; yeast extract (Difco), 2 g; agar (Nakarai, Kyoto, Japan), 15 g; pure water, 1000 ml; pH 5.5) and grown at 25.0° ± 0.5°C in the dark until a basidioma formed. The slant was then placed horizontally to force the basidiospores to be dispersed onto the inside surface of the test tube. Thereafter, the basidiospores were collected aseptically and suspended in sterile 100 mM $(NH_4)_2HPO_4$ aqueous solution to

induce germination (Suzuki et al. 1982). The spore suspensions were diluted several times by the same chemical solution followed by incubation for 1 h at 25.0° ± 0.5°C in the dark. The spore suspension was then spread over the surface of a plate medium [D-glucose, 1 g; L-sorbose, 1.5 g; agar (Nakarai), 15 g; pure water, 1000 ml] and incubated for 12–48 h at 25.0° ± 0.5°C in the dark. Then, we isolated cultures from single basidiospores. The monokaryotic isolates were confirmed by the absence of clamp connections after about 7 days of growth. The monokaryons used for the experiments reported in this article were assigned temporary numbers, CHU001–CHU012.

Mon–mon mating tests

The mating inoculation was conducted by plating plugs of two different monokaryotic stocks 5 mm apart in the center of a MY agar plate. After incubation for about 2 weeks at 25.0° ± 0.5°C in the dark, a piece of mycelium was collected from both the contact zone of two colonies and the outer edge of each colony on the line joining the centers of the two inoculum plugs. Thereafter, we identified compatible crosses as those having clamp connections formed throughout the paired colony. Individual pairings were performed three times. We selected monokaryotic isolates having different kinds of incompatibility factors as testers for the following di–mon mating tests.

Di–mon mating tests

The mating inoculation was conducted by plating one of the monokaryotic testers and each dikaryotic isolate 5 mm apart in the center of a MY agar plate. After incubating for about 2 weeks at 25.0° ± 0.5°C in the dark, a piece of mycelium was removed from the outer edge of the monokaryotic colony on the line joining the centers of the two inoculum plugs. Thereafter, we determined compatible crossings as those having clamp connections at an outer edge of the tester colony. Individual pairings were performed three times.

Results and discussion

Nucleotide sequences of the amplified nuclear ITS region and phylogenetic analysis

Nucleotide sequences of the amplified nuclear ITS region were determined for 26 cultures or basidiomata. ITS regions ranged in length from 711 to 714 bp in *Coprinopsis phlyctidospora*, from 715 to 720 bp in *C. echinospora*, and 725 bp in *C. strossmayeri*. Their sequences are deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB071608–AB071617 and AB071788–AB071803. Based on these aligned sequences, we constructed an unrooted molecular phylogenetic tree of three taxa of *Coprinopsis* species after 1000 bootstrap replications (Fig. 1).

In morphological studies, *C. phlyctidospora*, *C. echinospora*, and *C. strossmayeri* were classified as species in the genus *Coprinus* (Persoon ex Fries) S.F. Gray (1821), section *Coprinus* [*Comati* Fr. (1838) em. Lange (1915) or *Pelliculosi* (Fr. 1838 ut tribus) em. Schröter (1889)], subsection *Alachuani* Singer (1949); the former two taxa have warty basidiospores whereas the latter taxon has smooth basidiospores (Hongo and Yokoyama 1976; Hongo 1986; Uljé and Noordeloos 1997). Orton and Watling (1979) segregated a warty spore group (stirps *Echinosporus*) from smooth spore groups in section *Coprinus*. Thus, *Coprinopsis echinospora* and *C. strossmayeri* were examined as outgroups of *C. phlyctidospora*. The phylogenetic tree for the 26 sequences of tested fungal specimens was separated into four clades, i.e., two *C. phlyctidospora* groups, one *C. echinospora* group, and a *C. strossmayeri* isolate. The topology of the tree was well supported by high bootstrap values (see Fig. 1).

Coprinopsis phlyctidospora in clade A and the *C. echinospora* clade each included collections from various parts of Japan and a collection from the Netherlands, whereas *C. phlyctidospora* in clade B had collections from the North Island of New Zealand and from Dwellingup, southwestern Australia (Fig. 1; Table 1). The genetic distance within clade A was 0.0000 (e.g., CHU3004–CBM-FB21061) to 0.0099 [CHU3018–1026 (Uljé)], showing that variations among the samples from the Netherlands and those collected from different geographic parts of Japan are small. The difference between the specimen CBM-FB21220 from Hokkaido Island and the isolate CHU3018 from Kyushu Island is somewhat smaller than the genetic distance among isolates in clade B, which was 0.0000 to 0.0100. In clade B, the maximum values reflect the difference between the isolates CHU3016 and either CHU3013 or CHU3015 collected in the same plot in New Zealand. Both specimens from southwestern Australia are closely similar to the isolates CHU3002, CHU3007, CHU3009, CHU3013, CHU3014, CHU3015, and CHU3016 collected from four different sites in New Zealand (Fig. 1; Table 1).

In *C. echinospora*, the genetic distance among samples was from 0.0042 (IFO30631–CBM-FB21629) to 0.0317 (CBM-FB21264–CBM-FB21629). This fungus has more variation in the nuclear DNA ITS than the *C. phlyctidospora* in the two clades.

In the case of interclade variations, the genetic distance between clade A and clade B was 0.0217 and much greater than those among isolates in either clade A or clade B. The genetic distance between clade A and the *C. echinospora* clade was 0.0374 and that between clade B and the *C. echinospora* isolate was 0.0410. The genetic distances between either clades A or B and the *C. echinospora* clade was less than that between clades A or B and the *Coprinopsis strossmayeri* isolate (Fig. 1). These data obtained from the nucleotide sequences of ITS rDNA supported the conclusions reached about relationships among these fungi from morphological studies (Kühner and Romagnesi 1953;

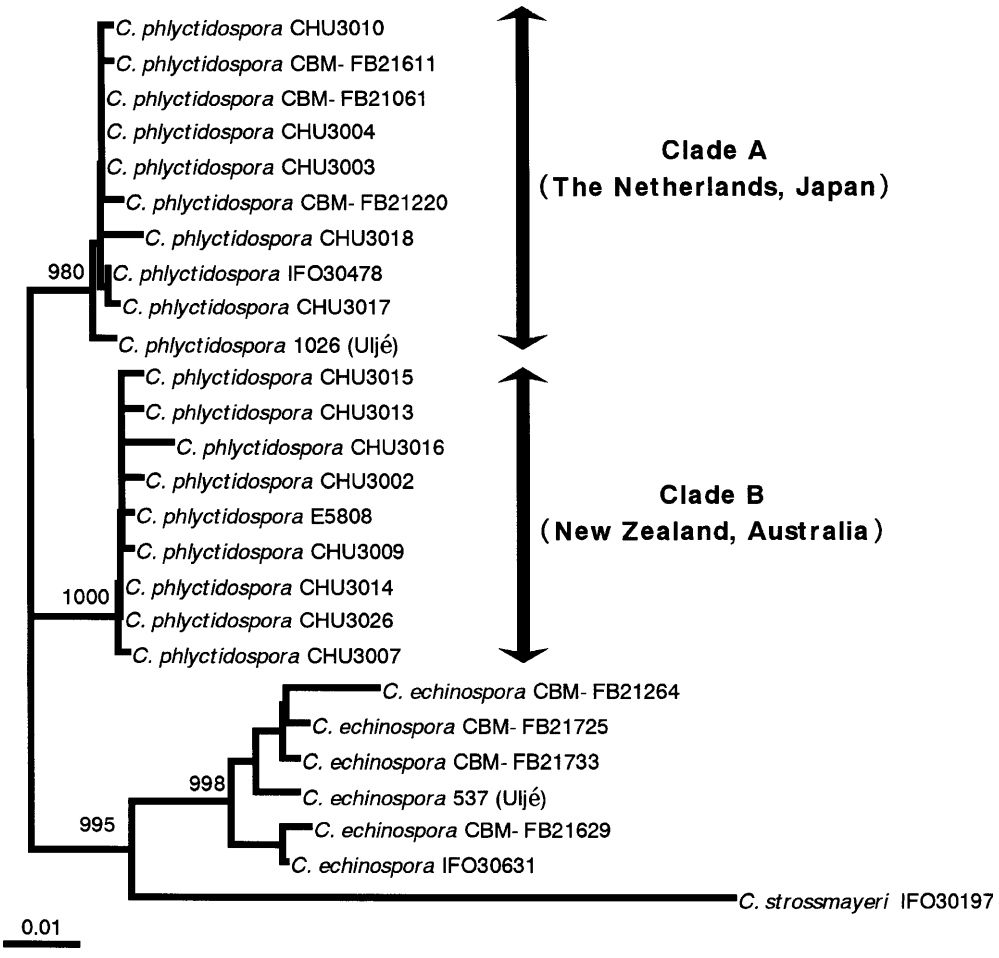

Fig. 1. Phylogenetic tree constructed by neighbor-joining method for 26 collections of *Coprinopsis* species based on nucleotide sequences of the ITS region. The values at the nodes are the confidence levels from 1000 replicate bootstrap samplings. The distance corresponding to 10 base changes per 1000 nucleotide positions is indicated by the *bar*

Moser 1978; Orton and Watling 1979; Hongo 1986; Uljé and Noordeloos 1997).

The level of genetic differentiation between two biogeographically diverse samples of *C. phlyctidospora* (clade A: the Netherlands – Japan; clade B: New Zealand – Australia) suggests that *C. phlyctidospora* is a complex of two allied taxa. The results also indicate that genetic variation within *C. echinospora* samples is greater than interclade variation of *C. phlyctidospora*. This result may imply that *C. echinospora* is also a species complex. However, further studies with more samples are needed to resolve this implication.

Mating type of *Coprinopsis phlyctidospora* in clade A

We observed clamp connections on dikaryotic hyphae of all tested vegetative mycelia as described by Leone (1978/1979b). We also observed that hyphae forming basidiomata have clamps as described by Aoki and Hongo (1965), although Orton and Watling (1979) reported the absence of clamps on hyphae. Pairings were designated as compatible when clamp connections formed on three parts of the parent mycelia as described here. The results indicate that the mating type of *C. phlyctidospora* (based on IFO30478) was tetrapolar, although pseudoclamps were not always seen in crosses between isolates having common B (Table 2). We therefore selected four monokaryotic isolates, i.e., CHU01, CHU04, CHU09, and CHU11, having different combinations of incompatibility factors, as the testers for subsequent mating tests.

Compatibility of intra- and interclade matings

The isolates of *C. phlyctidospora* obtained from a wide geographic area in Japan were completely compatible with all Japanese testers, although smaller clamp connections were observed in matings between dikaryotic isolates CHU3004 and CHU3007 and monokaryotic tester CHU11 at the outer colony edge on the line joining the centers of the two inoculum plugs (Table 3). In contrast, mating between the dikaryotic isolates collected in New Zealand and Australia and those of *C. phlyctidospora* collected in Japan were incompatible and behaved as different biological species *sensu* Brasier (1997) (Table 3). Dikaryotic *C. echinospora* was also incompatible with the *C. phlyctidospora* tester isolates. These results are consistent with the groupings based on their nuclear ITS sequences. The results of the phylogenetic and hybridization studies presented in this article revealed that *C. phlyctidospora* is a complex consisting of at least two taxa.

Table 2. Mating pattern among 12 monokaryotic isolates obtained from a basidioma produced in culture of *Coprinopsis phlyctidospora* IFO30478 from Japan

		A_1B_1			A_2B_2				A_1B_2		A_2B_1	
		CHU001	CHU002	CHU003	CHU004	CHU005	CHU006	CHU007	CHU008	CHU009	CHU010	CHU011	CHU012
A_1B_1	CHU001	−	−	−	+	+	+	+	+	−	−	−	(+)
	CHU002	−	−	−	+	+	+	+	+	−	−	−	−
	CHU003	−	−	−	+	+	+	+	+	−	−	−	−
A_2B_2	CHU004	+	+	+	−	−	−	−	−	−	−	−	−
	CHU005	+	+	+	−	−	−	−	−	−	(+)	−	−
	CHU006	+	+	+	−	−	−	−	−	(+)	−	−	−
	CHU007	+	+	+	−	−	−	−	−	(+)	(+)	−	−
	CHU008	+	+	+	−	−	−	−	−	−	−		−
A_1B_2	CHU009	−	−	−	−	−	(+)	(+)	−	−	−	+	+
	CHU010	−	−	−	−	(+)	−	(+)	−		−	+	+
A_2B_1	CHU011	−	−	−	−	−	−	−	−	+	+	−	−
	CHU012	(+)	−	−	−	−	−	−	−	+	+	−	−

+, clamp connections formed; (+), pseudoclamps formed; −, clamp connections did not form

Table 3. Results of dikaryon–monokaryon mating tests between dikaryotic isolates of *Coprinopsis phlyctidospora*, originating from Japan (clade A), New Zealand and Australia (clade B), and monokaryotic tester isolates of *C. phlyctidospora* originating from Japan (clade A)

Dikaryotic isolate	Tester[a]			
	A_1B_1 CHU001	A_2B_2 CHU004	A_1B_2 CHU009	A_2B_1 CHU011
C. phlyctidospora				
CHU3003[b]	+	+	+	+
CHU3004[b]	+	+	+	(+)
CHU3010[b]	+	+	+	+
CHU3017[b]	+	+	+	(+)
CHU3018[b]	+	+	+	+
CHU3002[c]	−	−	−	−
CHU3007[c]	−	−	−	−
CHU3009[c]	−	−	−	−
CHU3013[c]	−	−	−	−
CHU3014[c]	−	−	−	−
CHU3015[c]	−	−	−	−
CHU3016[c]	−	−	−	−
CHU3026[d]	−	−	−	−
C. echinospora				
IFO30631[b]	−	−	−	−

+, clamp connections formed; −, clamp connections did not form; (+), small clamp connections formed

[a] Testers were monokaryotic isolates of *C. phlyctidospora* IFO30478

[b] Dikaryotic isolate from Japan

[c] Dikaryotic isolate from New Zealand

[d] Dikaryotic isolate from Australia

Biogeographic distribution of *Coprinopsis phlyctidospora* complex

Coprinopsis phlyctidospora was first recorded on a forest floor of a burnt site with charcoal in Orlêans, France (Romagnesi 1945). In Japan, Aoki and Hongo (1965) identified a *Coprinopsis* species growing on vegetable manure heaps as *C. phlyctidospora* on the basis of morphological, macroscopic, and microscopic characters. *C. phlyctidospora* also has been recorded from Italy (Leone 1978/1979a), England (Orton and Watling 1979), and the Netherlands (Uljé and Noordeloos 1997). Orton and Watling (1979) described this fungus as "seemingly rare" and recorded it on burnt ground. In contrast, *C. phlyctidospora* has been found at high frequency after an application of a large amount of nitrogenous chemicals in various kinds of habitats, such as *Fagus*, *Castanopsis*, *Quercus*, *Pasania*, and *Pinus* forests, *Chamaecyparis*, *Aphanante-Ulmus*, and *Phyllostachys* stands, and a few weed communities, in different geographic locations of Japan (Sagara 1975; Suzuki 1992; Fukiharu and Hongo 1995; Fukiharu and Horigome 1996; Fukiharu et al. 1997; Sato and Suzuki 1997; Suzuki and Toyokawa 1998/1999; He and Suzuki 2000). The difference in the frequency of occurrence of this fungus may reflect its habitat, i.e., it is usually observed on soils disturbed by fire (Romagnesi 1945; Orton and Watling 1979) or nitrogenous materials (Sagara 1975, 1992).

In the Southern Hemisphere, *C. phlyctidospora* had been recorded only once, from rotted basal stem tissue of the passion vine *Passiflora edulis*, near Perth in southwestern Australia (Doepel 1968) before we started to apply urea in

the forests in New Zealand and Australia. It was speculated that *C. phlyctidospora* may have occurred through tissue injured by application of ammonia fertilizer or through wounds (Doepel, 1968). One of the authors of this present paper (Fukiharu) reexamined morphological characters of the specimen of *C. phlyctidospora* collected by Doepel (1968) and deposited in the Royal Botanical Garden, Kew. He concluded that it was different from the *C. phlyctidospora* in clade B as well as from *C. phlyctidospora* in clade A collected from Europe and Japan. We collected samples of the *C. phlyctidospora* representing clade B after a large amount of urea was applied to soil in native forests in the Southern Hemisphere. The forests include beech–rimu forest (dominated by *Nothofagus fusca* mixed with *Nothofagus menziesii* and *Dacrydium cupressinum*) and beech forest (dominated by *N. fusca* mixed with *N. menziesii*) in Taupo, New Zealand (see Table 1) and a eucalyptus forest (dominated by *Eucalyptus marginata* and *E. calophylla*, with smaller trees, e.g., *Allocasuarina* and *Banksia* and a diverse plant understory) near Perth in Australia (Table 1). *C. phlyctidospora* in clade B was also collected from a plantation of *Pinus radiata*, an introduced species, in River-head, Auckland, New Zealand (Table 1). The genetic variation between the isolates obtained from the native forests in New Zealand and the isolate obtained from the *P. radiata* stand in New Zealand was negligible, which suggests that the native *C. phlyctidospora* in clade B has colonized the introduced tree plantation (see Fig. 1). The biogeographic distribution of ammonia fungi can be assessed with greater precision by application of urea on the surveying sites at appropriate times.

Based on the definition of ammonia fungi by Sagara (1975), not only *C. phlyctidospora* in clade A but also *C. phlyctidospora* in clade B belongs to this chemoecological group. In Japan, *C. phlyctidospora* in clade A has been collected at high frequency in the urea-treated plots and from the animal waste sites, but *C. phlyctidospora* in clade B has not been recorded at these same places (Sagara 1975; Suzuki 1992; Sagara 1995; Fukiharu and Hongo 1995; Fukiharu and Horigome 1996; Fukiharu et al. 1997; Sato and Suzuki 1997; Suzuki and Toyokawa 1998/1999; He and Suzuki 2000). In contrast, *C. phlyctidospora* in clade A has not been observed in the many urea-treated plots in New Zealand and near Perth in southwestern Australia, despite the high frequency of occurrence of clade B *C. phlyctidospora* in the same places (unpublished observation). These results suggest that *C. phlyctidospora* in clade A has not yet invaded these sites in the Southern Hemisphere. The intraspecific variances within each *C. phlyctidospora* clade were small in spite of their wide distributions in each hemisphere (Fig. 1), which supports the idea that *C. phlyctidospora* in clades A and B occupy similar niches in predominantly Laurasian and Gondwanan vegetations, respectively.

Lange (1952) found a similarly wide geographic distribution for other "*Coprinus* species *sensu lato*" and concluded that the entire flora (= fungus species assemblage) of coprophilous *Coprinus* species *sensu lato* was universally distributed, at least in the northern temperate zone. This conclusion was derived from the interfertility of the heterothallic strains of coprophilous *Coprinus* species *sensu lato* and strains of a pyrophilous fungus, *Coprinellus angulatus* (Peck.) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus angulatus* Peck.), which were collected from different geographic areas, and from the identification of the cultural characters of homothallic *Coprinus* species *sensu lato*. Bougher (1983) also suggested that coprophilous *Coprinus* species *sensu lato* were ubiquitous because there was no definitive difference in morphological characters between specimens from Europe and from Western Australia and because of their nonspecific preference to substrates. Hongo (1978) categorized the biogeographic distribution of Agaricales found in Japan into nine types. In their paper, they referred to the worldwide distribution of coprophilous fungi, such as *Stropharia semiglobosa* (Batsch: Fr.) Quél., having long-lived pigmented basidiospores, and to the extension of the distribution area of *Panaeolus papillonaceus* (Bull.: Fr.) Quél. and *Coprinopsis cinerea* (Schaeff.: Fr.) Redhead, Vilgalys & Moncalvo (syn.: *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray) in recent time with human migrations.

The mechanisms for dispersal of the *C. phlyctidospora* complex have not been investigated in relation to the effectiveness of spore dispersal over long distances and time. *C. phlyctidospora* in clade A established in bottles containing γ-ray-sterilized urea-treated soils, which had been exposed for 2 weeks on a desk, and placed in a mixed forest in Japan (Suzuki 1992). This experiment indicated that *C. phlyctidospora* in clade A can propagate into noninvaded places through the air as well as through the ground. In controlled laboratory conditions, cultures of *C. phlyctidospora* in clade A can sustain high fruiting abilities for long periods of time (e.g., *C. phlyctidospora* IFO30478, isolated in 1977 and subcultured more than 70 times), and its basidiospores are easy to germinate when they are treated with urea or ammonium salts (Suzuki et al. 1982). *C. phlyctidospora* in both clades A and B have pigmented basidiospores, but there are no data concerning their viability over long periods of time. These findings suggest that the distribution areas of the two *Coprinopsis* species are separated by indiscernible barriers.

Uljé and Noordeloos (2000) proposed that *Coprinus lagopides* P. Karst. [now *Coprinopsis lagopides* (P. Karst.) Redhead, Vilgalys & Moncalvo] collected and named by Karsten (1879) is identical to, and has nomenclatural priority over, *Coprinopsis phlyctidospora*, which was first named by Romagnesi (1945) as *Coprinus phlyctidosporus*. Uljé and Noordeloos (2000) also pointed out that a later collection by Karsten in 1884 and labeled by him as *C. lagopides* is not the same as the 1879 collection and appears to conform to a misapplied concept of *C. lagopides*. The correct identity of that collection is *Coprinus jonesii* Peck, now, according to Redhead et al. (2001), *Coprinopsis jonesii* (Peck) Redhead, Vilgalys & Moncalvo. For this article, we opted to retain usage of the name *C. phlyctidospora* rather than *C. lagopides* on the basis of the redescription by Uljé and Noordeloos (2000). It is not possible to confirm whether the specimen described by Karsten (1879) matches the species concept of *C. phlyctidospora* as defined by Romagnesi

(1945). Some doubt also remains because of the poor state of the type material studied by Uljé and Noordeloos (2000). Also, in view of the age and poor condition of the type material of *C. lagopides*, it may not be possible to elucidate molecular data for that fungus.

Further collections of these fungi induced by disturbance and subsequent analyses of their compatibility groups and molecular, morphological, and physiological characters are required to elucidate the extent of variation within the *C. phlyctidospora* complex in relation to its biogeographic distribution. Moreover, further collections over a broader geographic range may help to resolve the relationship of this complex to *C. lagopides*.

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