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SHORT COMMUNICATION

Coprinopsis austrophlyctidospora sp. nov., an agaric ammonia fungus from Southern Hemisphere plantations and natural forests

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Abstract A new ammonia fungus, *Coprinopsis austrophlyctidospora*, is described from *Nothofagus* and *Pinus* forests in New Zealand and from *Eucalyptus* forest in Australia. In ecology and macro-morphology, this species is similar to the Northern Hemisphere species *C. phlyctidospora*, but the new species differs in morphological characters of the basidiospore, i.e., in having a plage, more minute surface warts, and the smaller size of the basidiospore.

Keywords Agaricales · Australia · *Coprinus phlyctidosporus* · New Zealand

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Application of urea to soil stimulates the occurrence of an ecological group of fungi called 'ammonia fungi' (Sagara 1975, 1992). In the course of our study of ammonia fungi, we investigated the ammonia fungi of the Southern Hemisphere during 1991–2009. From *Nothofagus* and *Pinus* forests in New Zealand and from *Eucalyptus* forest in Australia, *Coprinopsis phlyctidospora* (Romagn.) Redhead, Vilgalys & Moncalvo (Syn.: *Coprinus phlyctidosporus* Romagn.) sensu lato was collected in each forest following urea treatment (Suzuki et al. 2002). *Coprinopsis phlyctidospora* sensu lato from The Netherlands, Japan, New Zealand, and Australia was segregated into two distinct taxa, the Northern Hemisphere group and Southern Hemisphere group, based on nucleotide sequences of their ITS regions and on the results of mating tests (Suzuki et al. 2002), but morphological differences were not elucidated. We have therefore examined the macro- and microscopic characters of both taxa to determine the taxonomic position of the Southern Hemisphere group and to describe it as a new species.

Field observations were conducted in three forests: *Nothofagus menziesii* (Hook.f.) Oerst. and *Nothofagus fusca* (Hook.f.) Oerst. dominated forests in Kaimanawa State Forest Park, Taupo, North Island, New Zealand (NZ); a *Pinus radiata* D.Don planted forest in Riverhead, North Island, NZ; and a *Eucalyptus marginata* Donn ex Smith and *Corymbia calophylla* (Lindl.) K. D. Hill & L. A. S. Johnson dominated forest in Dwellingup, near Perth, Western Australia, Australia (Table 1, Suzuki et al. 2002). In the field experiments, urea (granular fertilizer; 46% nitrogen) was applied to the ground surface in each forest at the rate per square meter of 800 g (in Australia), 696 g (in Taupo, NZ), and 348 g (in Riverhead, NZ), to stimulate fruiting of ammonia fungi. In the laboratory, urea (aqueous fertilizer; 20 mg N/g dry weight soil) was applied to soil and incubated at 20°C under a 12 h light/12 h dark lighting regime. Humidity was sufficient

Table 1 Study sites, specimens and cultures of *Coprinopsis austrophlyctidospora*

	Locality of collection	Vegetation	Plot size (m)	Urea application date	Treatment[b]	Isolation and collection date	Specimen no.	Isolate no.[a]
Australia	Dwellingup, near Perth, Western Australia	*Eucalyptus marginata*, *Corymbia calophylla* dominated forest	1 × 2	8 May 1997	Field (800 g)	14 July 1997	FB-24556 (CBM)	CHU3026
			1 × 2	18 July 1996	Field (200–800 g)	19 September 1996	PERTH 07598351	
							PERTH 07599137	
New Zealand	Kaimanawa State Forest Park, Taupo, North Island	*Nothofagus menziesii*, *Nothofagus fusca* dominated forest	1 × 0.5	9 March 1994	Field (696 g)	20 November 1994	PDD 76873 (Holotype); FB-24558, 30231, 30236, 30238, 30240 (CBM)	CHU3002
			1 × 0.5	21 May 1993	Field (696 g)	22 March 1994	FB-24568 (CBM)	CHU3009
			–	19 November 1994	Laboratory	8 June 1995	FB-24562 (CBM)	CHU3013
	Riverhead, North Island	*Pinus radiata* (plantation)	1 × 0.5	6 June 1991	Field (348 g)	18 September 1991	FB-24554 (CBM)	CHU3007

[a] Isolates indicated by CHU numbers are stock cultures of the Faculty of Education, Chiba University, Japan

[b] Weights shown in parentheses denote the amount of urea (granular form fertilizer; 46% nitrogen) spread per square meter in the field experiment. In the laboratory, aqueous urea was added to each soil at the rate of 20 mg (nitrogen)/g dry weight soil

to support fungal fruiting. Basidiomata observed in the field and in the laboratory were collected, and cultures were isolated from each basidioma. Cultures were grown at 25°C under a 12 h light/12 h dark lighting regime on MYC medium (Malt extract, Yeast extract, and Casamino acid; DIFCO) for about 2–3 weeks. Anatomical observations and measurements were made on material mounted in 25% aqueous ammonia. For scanning electron microscope (SEM) observation of basidiospore ornamentation, a small portion of a dried specimen was rehydrated in 25% aqueous ammonia, fixed in osmium acid, 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, with measurements of basidiospores from spore prints. Color notation used in the species description is according to Kornerup and Wanscher (1978). Specimens examined are deposited in the New Zealand Fungal Herbarium (PDD), Western Australian Herbarium (PERTH), and Natural History Museum and Institute, Chiba (CBM).

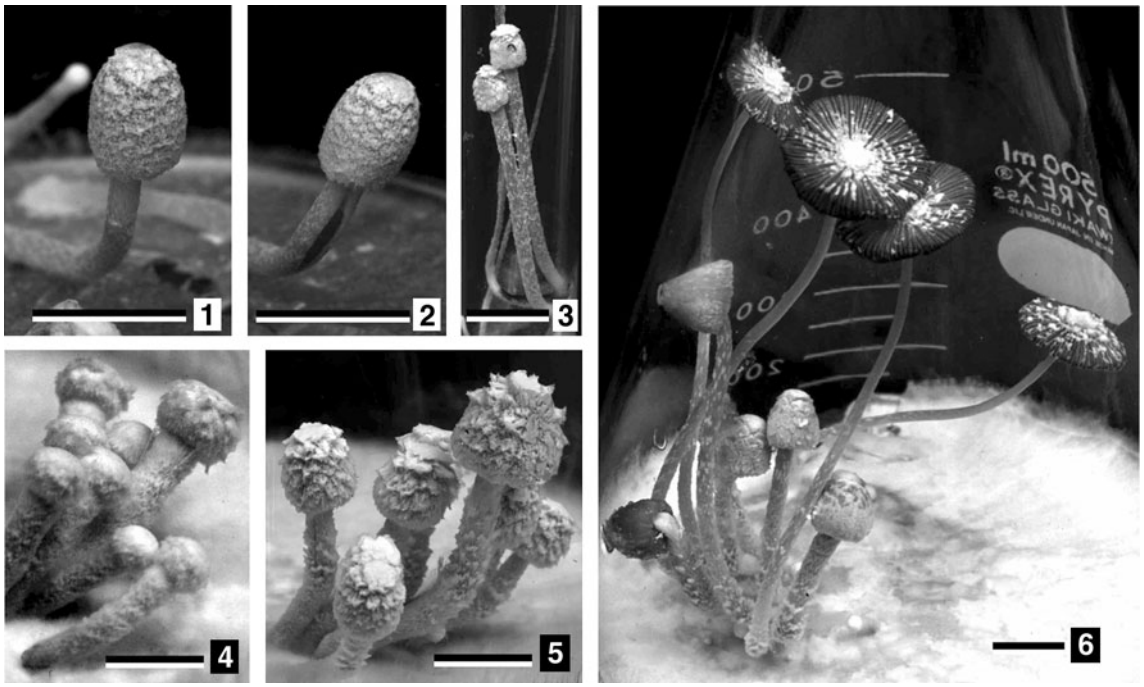
Coprinopsis austrophlyctidospora Fukiharu, sp. nov.
Figs. 1–9

MycoBank no.: MB 518948

Pileo primo 5–10 mm lato 6–12 mm alto, usque ad 15–20 mm lato, ovato-campanulato, demum in margine lacerato revolutoque, radiatim sulcato, candido vel eburneo, deinde cinerascenti, primo squamis albis radiatim hirsuto-fibrillosis squarrosis interdum recurvis ubique tecto, dein fere glabro, interdum primo cortinato; carne tenuissima, fragilissima, alba; sapore miti; odore nullo; lamellis liberis, comfertis, angustis (1–2 mm), albis, deinde atratis, deliquescentibus; stipite 50–100 mm longo, 1–3 mm crasso, aequali vel sursum leviter attenuato, basi leviter incrassatulo, cavo, candido, fragilissimo, primo squamis albis fibrillosis squarrosis ubique tecto, dein glabro; basidiosporis in cumulo atratis, sub microscopio atro-brunneis vel rufo-brunneis, ovoideis vel ellipsoideis, 6.5–7.5 × 5.1–6.5 (frontali) × 5.1–6.2 (laterali) µm, verrucosis, poro germinationis centrali 0.7–0.8 µm lato, cum plagiis; basidiis 14–25 × 6–7.5 µm, tetrasporis; pleurocystidiis 65–80 × 40–50 µm, subellipsoideis vel obovatis, hyalinis, tenuitunicatis; cheilocystidiis 35–50 × 25–30 µm, subellipsoideis vel obovatis, hyalinis, tenuitunicatis; velo pilei ex hyphis tenuitunicatis, divaricatis, hyalinis, 50–150 × 4–6 µm composito; fibulis praesentibus.

Holotypus: New Zealand, leg. T. Fukiharu, in Herbario Fungorum Novae Zelandiae conservatus (PDD 76873).

Etymology: The Latin *austro-* (Southern) refers to the Southern Hemisphere origin of this species, and



Figs. 1–6 *Coprinopsis austrophlyctidospora*. Cultured at 25°C, 17–23 days with MYC medium. **1**, **2** FB-30238 (CBM). **3** FB-30240 (CBM). **4**, **6** PDD76873 (holotype). **5** FB-30231 (CBM). *Bars* 10 mm

-phlyctidospora reflects the morphological resemblance of this species to *C. phlyctidospora*.

Pileus 5–10 mm broad, 6–12 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, soon becoming brown (6d6), surface when young densely covered with white, radially arranged, hairy-fibrillose scales (Figs. 1, 2) or squarrose recurved scales (Figs. 4, 5), sometimes with cortinate veil in very young stage (Fig. 4), later almost glabrous or veil remaining only in the center (Fig. 6). Flesh very thin, fragile, white, taste mild, odorless. Lamellae free, crowded (number of lamellae reaching stipe = 70–85), narrow (1–2 mm wide), edge slightly pruinose, at first white, then grayish, finally blackish, deliquescent. Stipe up to 50–100 mm × 1–3 mm, cylindrical, equal or somewhat tapering upward, sometimes the base clavate, not rooting, hollow, fragile, surface white, at first with white fibrillose or squarrose scales (Figs. 3, 4, 5), soon becoming smooth (Fig. 6). Basidiospores black in mass, dark red-brown under the microscope, ovoid to ellipsoid, 6.5–7.5 (7.0 ± 0.3: mean ± SD, $n = 40$) µm long, 5.1–6.5 (5.7 ± 0.4, $n = 20$) µm broad in face view, 5.1–6.2 (5.6 ± 0.3, $n = 20$) µm in side view (dimensions including ornamentation); 6.2–6.8 (6.6 ± 0.3, $n = 40$) µm long, 4.9–6.0 (5.4 ± 0.3, $n = 20$) µm broad in face view, 4.8–5.6 (5.3 ± 0.2, $n = 20$) µm in side view (dimensions without ornamentation), with warty ornamentation, an apical, central germ pore 0.7–0.8 µm wide and a clear plage (Figs. 7a, 8, 9). Basidia 14–25 × 6–7.5 (long type: 20–25 × 6–7.5, short type: 14–18 × 6–7) µm, 4-spored (Fig. 7b). Pleurocystidia 65–80 × 40–50 µm, subellipsoid to obovoid, thin-walled, hyaline, scattered and projecting from the hymenium (Fig. 7c). Cheilocystidia 35–50 × 25–30 µm, subellipsoid to obovoid, thin-walled, hyaline, numerous, but not forming sterile margin (Fig. 7d). Veil on the pileal surface composed of thin-walled, diverticulate, hyaline hyphae, 50–150 × 4–6 µm (Fig. 7e). Clamp connections present on veil hyphae (Fig. 7e), on pleuro- and cheilocystidia hyphae (Fig. 7c, d) and on pileal trama hyphae.

Specimens examined: *Coprinopsis austrophlyctidospora*: All specimens for descriptions were cultivated from the same field-collected stock culture (Table 1, CHU3002: Suzuki et al. 2002), isolated from urea-treated plots in *Nothofagus* forest, Taupo, New Zealand: PDD 76873—Holotype; FB-30231, FB-30236, FB-30238, FB-30240 (CBM); FB-24558 (spore print, CBM). Specimens produced from other stock cultures from Taupo: FB-24562, 24568 (CBM); from Riverhead, New Zealand: FB-24554 (CBM); from Dwellingup, Australia: FB-24556 (CBM). Field collected materials were also examined; from Dwellingup, Australia, PERTH 07598351 and PERTH 07599137 (Table 1).

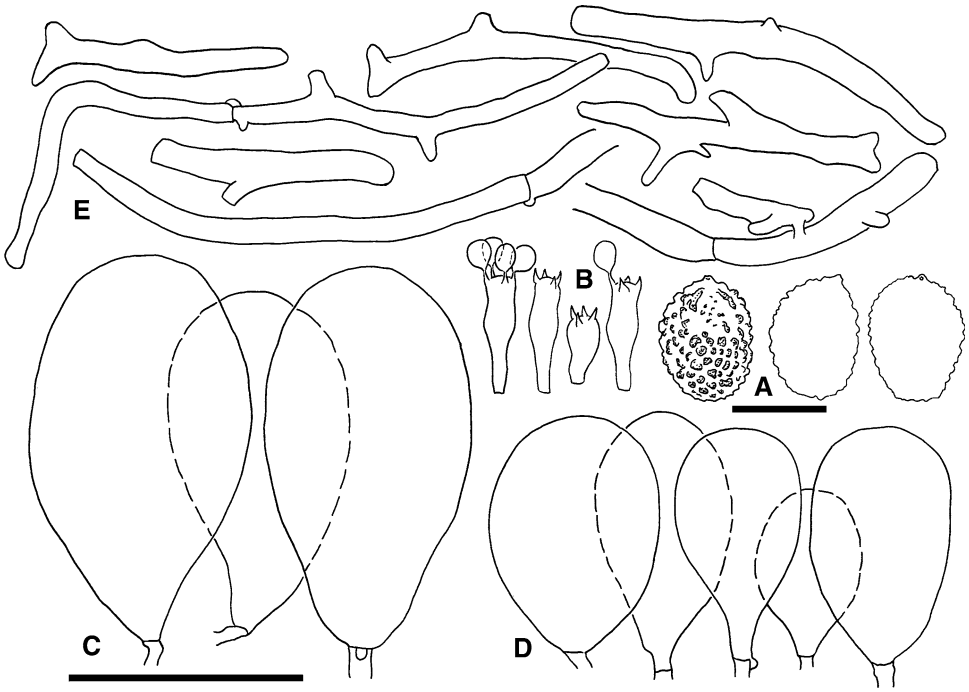
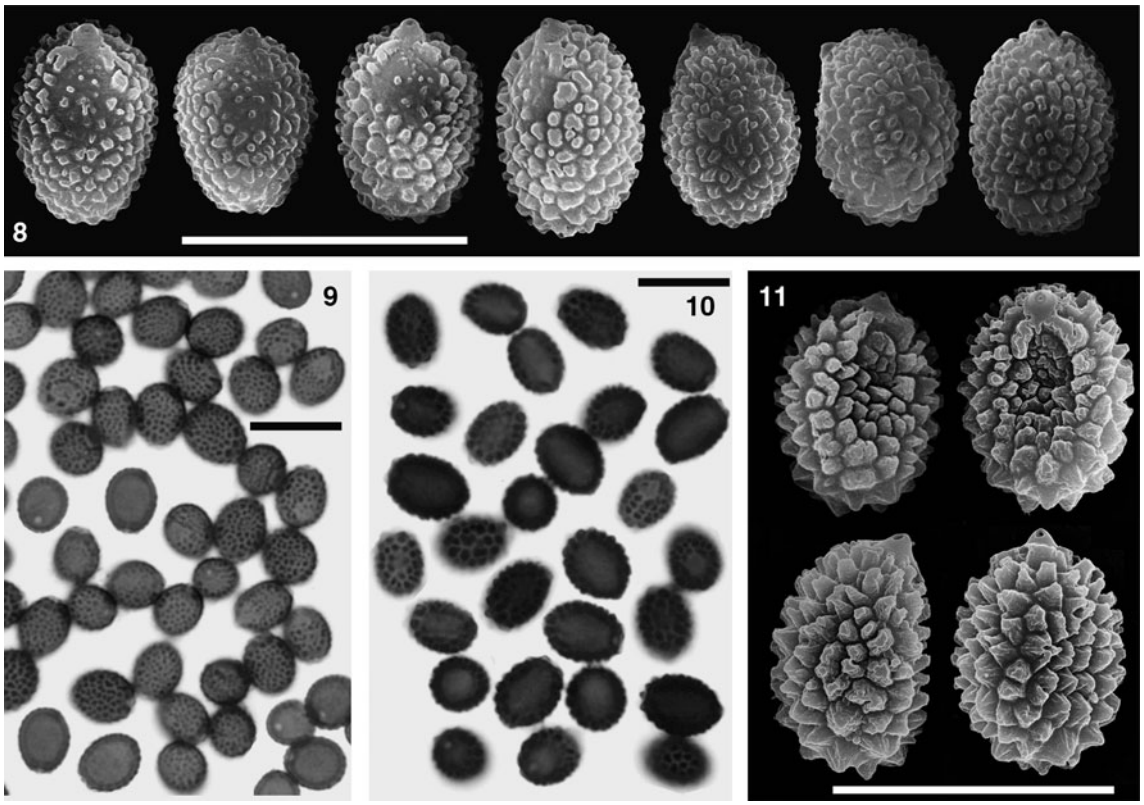
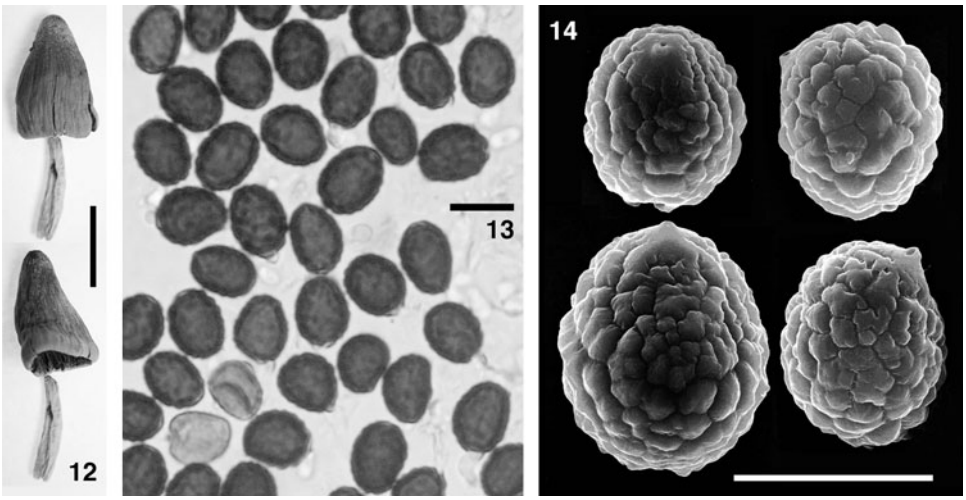


Fig. 7 *Coprinopsis austrophlyctidospora*. **a** Basidiospores (face view, side view, face view). **b** Basidia with basidiospores. **c** Pleurocystidia. **d** Cheilocystidia. **e** Veil hyphae. **a** from FB-24558 (CBM); **b–e** from FB-30240 (CBM). *Bars* **a** 5 μm; **b–e** 50 μm



Figs. 8–11 8, **9** Basidiospores of *Coprinopsis austrophlyctidospora*. From spore print of cultured basidiomata, FB-24558 (CBM). *Bars* 10 μm. **10**, **11** Basidiospores of *Coprinopsis phlyctidospora*. From basidioma, Herb. Lugd. Bat. No. 989.300 068, The Netherlands. *Bars* 10 μm



Figs. 12–14 *Coprinopsis* sp. The specimen collected from near Perth in southwestern Australia as *Coprinus phlyctidosporus* [Doepel 1968, M-85694 (Kew)]. Spore size is larger than in *C. austrophlyctidospora* and *C. phlyctidospora*. *Bars* **12** 10 mm; **13**, **14** 10 μm

Habit and habitat: Solitary to gregarious, appearing 2–10 months after urea treatment in *Nothofagus*, *Pinus*, and *Eucalyptus* dominated forests (Table 1). This fungus was observed at high frequency in each urea-treated plot (Suzuki et al. 2002), indicating that it is an ammonia fungus (Sagara 1975, 1992).

Distribution: New Zealand (Kaimanawa State Forest Park, Taupo, North Island; Riverhead, North Island), Australia (Dwellingup, Western Australia) (Suzuki et al. 2002).

Other specimens examined: *Coprinopsis phlyctidospora*: Herb. Lugd. Bat. No. 989.300 068, The Netherlands, No. 1026 (C.B. Uljé), 3 September 1989, Langeraar, Ter Aar, prov. Zuid-Holland, The Netherlands (Uljé and Noordeloos 1997). *Coprinopsis* sp.: M-85694 (Kew), from rotted basal stem tissue of passion vine *Passiflora edulis*, Lesmurdie, near Perth in southwestern Australia.

Notes. According to traditional *Coprinus* Pers. taxonomy, this species belongs to 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 there are three species reported to have warty ornamented basidiospores, i.e., *Coprinopsis echinospora* (Buller) Redhead, Vilgalys & Moncalvo (Syn.: *Coprinus echinosporus* Buller), *Coprinopsis phlyctidospora* (Syn.: *Coprinus phlyctidosporus*), and *Coprinopsis rugosobispora* (J. Geesink & Imler) Redhead, Vilgalys & Moncalvo (Syn.: *Coprinus rugosobisporus* J.Geesink & Imler) (Orton and Watling 1979; Moser 1983; Uljé and Noordeloos 1997; Uljé 2005). *Coprinopsis rugosobispora* is distinguished from *C. austrophlyctidospora* in having two-spored basidia. *Coprinopsis echinospora* differs in having amygdaliform basidiospores and two types of hyphae in the pileal veil. Morphologically, *C. austrophlyctidospora* is very similar to *C. phlyctidospora*, but the latter species has larger basidiospores (range: 7.5–11.0 × 5.5–8.0 μm, average: 8.4–10.6 × 6.0–7.6 μm, Uljé and Noordeloos 1997; Uljé 2005) that are more oblong shaped and have a more coarsely warted ornamentation than those of *C. austrophlyctidospora* (Figs. 10, 11). *Coprinopsis phlyctidospora* has more distinct warts surrounding the germ pore. The plage of *C. phlyctidospora* is sometimes not as distinct as that of *C. austrophlyctidospora* (Figs. 8, 9). The basidiospore color of *C. phlyctidospora* under the microscope is darker brown. These morphological differences between the two species support segregation of *C. phlyctidospora* sensu lato into two distinct taxa, one northern and the other southern, as first indicated from rDNA ITS nucleotide sequences and results of mating tests (Suzuki et al. 2002). Orton and Watling (1979) reported that clamp connections were not observed in *C. phlyctidospora*. In the present study, clamp connections were observed for both species on hyphae of the pileal veil of cultivated basidiomata. Suzuki et al. (2002) prepared mating tests between these two species with matings indicated by clamp connections. Thus, clamp connections are known to exist in cultivated mycelium and now also in basidiomata of both species. The description above is based on cultivated material from New Zealand. Field collected specimens (PERTH 07598351 and PERTH 07599137, Australia, Table 1) have similar morphological characters as cultivated specimens, both macro- and microscopically. Basidiospores of the Australian specimens (not from spore print but from lamellae of mature basidiomata) are: 6.3-7.4 (6.9 ± 0.4: mean ± SD, $n = 40$) μm long, 5.1–6.4 (5.7 ± 0.3, $n = 20$) μm broad in the face view, and 5.1–6.3 (5.6 ± 0.4, $n = 20$) μm in the side view (including ornamentation). From Australia, "*Coprinus phlyctidosporus*" auct. non Romagn. was previously

recorded only once, from rotted basal stem tissue of passion vine *Passiflora edulis* Sims, near Perth in southwestern Australia (Doepel 1968; Hilton 1982). The specimen reported by Doepel [M-85694 (Kew), Figs. 12, 13, 14] is similar to both *C. austrophlyctidospora* and *C. phlyctidospora* in having diverticulate pileal veil hyphae and warty ornamented basidiospores. But the basidiospores of Doepel's specimen are large, i.e., 11.0–14.5 (13.0 ± 0.9: mean ± SD, $n = 40$) μm long, 8.5–11.6 (10.0 ± 0.7, $n = 20$) μm in the face view, and 9.2–11.0 (10.0 ± 0.8, $n = 20$) μm in the side view (Figs. 13, 14), and basidiomata are also more stout and large (pileus up to 14 mm high, Fig. 12). This specimen seems to be a different and undetermined species of *Coprinopsis*. Another *Coprinopsis* species with warted basidiospore ornamentation reported from South Australia is *Coprinopsis karwinicola* (Grgur.) J.A. Simpson & Grgur. (Syn.: *Coprinus karwinicola* Grgur.) (Grgurinovic 1997). This species is distinguished from *Coprinopsis austrophlyctidospora* in having larger basidiomata (pileus up to 62 mm wide), larger basidiospores (10.4–13.4 × 7.2–8.6 μm) and in lacking cheilocystidia. Ecologically, *C. karwinicola* is not an ammonia fungus, having been collected on dead tissue of the grass tree *Xanthorrhoea* sp. (Grgurinovic 1997).

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