

SHORT COMMUNICATION

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Chlamydospore formation of *Entoloma clypeatum* f. *hybridum* on mycorrhizas and rhizomorphs associated with *Rosa multiflora*

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Abstract Chlamydospores of *Entoloma clypeatum* f. *hybridum* were described on the mycorrhizas and rhizomorphs associated with *Rosa multiflora*. Their developmental pattern seems to be the *Nyctalis* type. This is the first report on chlamydospore formation on the mycorrhizae in entolomatoid fungi.

Key words Basidiomycetous mushroom · Chlamydospore · *Entoloma clypeatum* f. *hybridum* · Mycorrhiza · *Rosa multiflora*

The term chlamydospore is defined as “an asexual one-celled spore (primary for perennation, not dissemination in survival strategy) originating endogenously and singly within part of a pre-existing cell, by the contraction of the protoplast and possessing an inner secondary and often thickened and hyaline or brown wall, usually impregnated with hydrophobic material” (Hawksworth et al. 1995). Various groups of fungi are known to form this type of spore on fruiting bodies and cultured mycelia (Hughes 1985). Recently, chlamydospore formation on the ectomycorrhizas have been described in various taxa, i.e., Elaphomycetaceae, Cortinariaceae, Gomphidiaceae, and Thelephoraceae (Agerer 1995), Boletaceae (Eberhart and Luoma 1996), and Tricholomataceae (Simazono 1979; Terashima et al. 1993; Lefevre and Müeller 1998; Gill et al. 1999; Yamada et al. 1999). These chlamydospores are unique in shape depending on the taxa, therefore having importance in characterizing and identifying the mycorrhizal morphotype (Agerer 1987–1998; Agerer 1996; Goodman et al. 1996–1999).

During the course of studies on the mycorrhizal association between entolomatoid fungi and rosaceous plants (Kobayashi and Hatano 2001), pinkish mycelia were observed around rhizomorphs and mycorrhizas of *Entoloma clypeatum* (L.) kumm. f. *hybridum* (Romag.) Noordel. associated with *Rosa multiflora* Thunb. (Fig. 1). The specimen was collected at Mt. Tatsuta, Kumamoto Prefecture, Japan (32°49' N, 130°45' E) on April 7, 1996. Rhizomorphal connections with fruiting bodies were traced to identify the colored mycelia. Spores were abundantly present in the mycelia. They were thick walled with roughened surface, ellipsoid with marginal segments, 12–16 × 5–7 μm (including segments), and hyaline to pinkish color (Fig. 2). Hyaline, rough-surface, and swollen cells were terminally observed in vegetative hyphae with clamp connections (Fig. 3). The surface view was the same for the swollen cells and the spores. Two spores arranged in a chain were also observed (Fig. 4). Fragmented clamp connections were observed on several hyphal tips (Fig. 5). Dried fruiting bodies, dried soil blocks containing spores, and mounted spores in slides examined were deposited in Osaka Museum of Natural History, Japan (OSA-My-5000).

Figures 3–5 suggest the spore formation to be thallic from the vegetative hyphal cells. Morphological features and the possible ontogeny of the spores agree with the definition of the chlamydospore (Hawksworth et al. 1995).

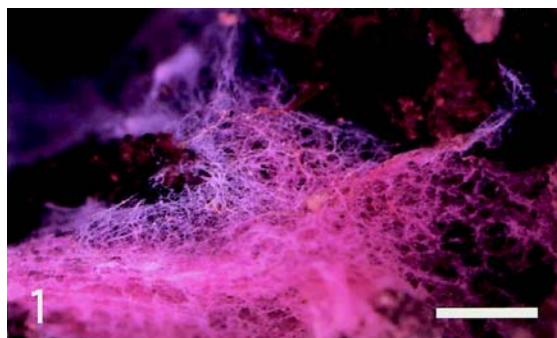


Fig. 1. Pinkish mycelium of *Entoloma clypeatum* f. *hybridum*. Bar 1 mm

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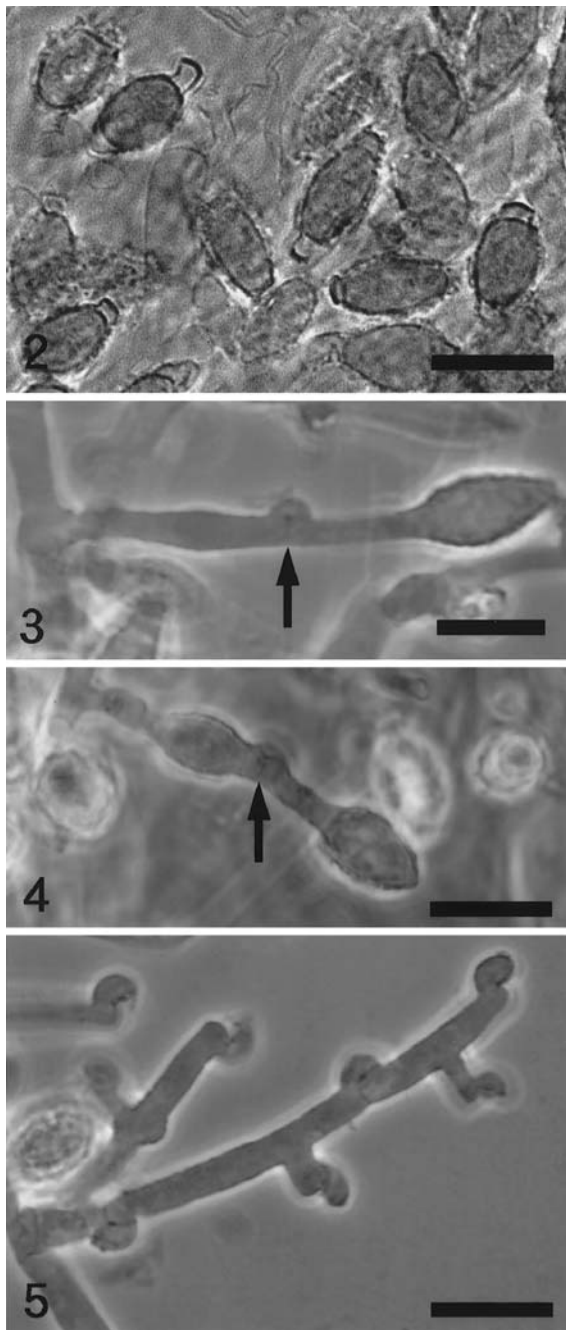


Fig. 2–5. 2 Light micrograph of chlamydospores of *E. clypeatum* f. *hybridum*. Bar 10 μ m. 3–5 Phase-contrast micrographs of *E. clypeatum* f. *hybridum* mycelium. Bars 10 μ m. 3 Clamped hyphae developing a chlamydospore terminally. Arrow, clamp connection. 4 A chain of two chlamydospores. Arrow, clamp connection. 5 Fragmented clamp connections on the hyphal tips

Furthermore, the marginal segment of the spore (Fig. 2) suggests formation from the external cell wall of the pre-existing swollen cell by secondary development of the inner cell wall, i.e., enterothallic-like development of the spore (Ulloa and Hanlin 2000). Kendrick and Watling (1979) divided the chlamydospore into six types in basidiomycetes based on the ontogeny. Chlamydospores in the present study belong to the *Nyctalis* type (Kendrick and Watling 1979).

Among Entolomataceae, similar chlamydospores were reported from a saprotrophic undescribed *Rhodocybe* species on agar plates (Baroni and Carey 1994), but this is the first report on chlamydospore formation in entolomatoid fungi on mycorrhizas.

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References

- Agerer R (ed) (1987–1998) Colour atlas of ectomycorrhizae, 1st–11th edn. Einhorn, Schwäbisch Gmünd
- Agerer R (1995) Anatomical characteristics of ectomycorrhizae: an attempt towards a natural classification. In: Varma AK, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 685–734
- Agerer R (1996) Characterization of ectomycorrhizae: a historical overview. *Descript Ectomycorrhizae* 1:1–22
- Baroni TJ, Carey P (1994) An undescribed anamorph of undescribed species of *Rhodocybe* (Agaricales, Entolomataceae). In: 5th International Mycological Congress, Vancouver, Canada, August 14–21, p 10
- Eberhart J, Luoma D (1996) Description code 9. *Truncocolumella citrina* Zeller + *Pseudotsuga menziesii* (Mirb.) Franco. In: Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) Concise descriptions of north American ectomycorrhizae. Mycologue Publications and the Canada-BC Forest Resource Development Agreement, Pacific Forestry Centre, Victoria
- Gill WM, Lapeyrie F, Gomi T, Suzuki K (1999) *Tricholoma matsutake*: an assessment of in situ and in vitro infection by observing cleared and stained whole roots. *Mycorrhiza* 9:227–231
- Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) (1996–1999) Concise descriptions of north American ectomycorrhizae. Mycologue Publications and the Canada-BC Forest Resource Development Agreement, Pacific Forestry Centre, Victoria
- Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Ainsworth & Bisby's dictionary of the fungi, 8th edn. CAB international, Wallingford
- Hughes SJ (1985) The term chlamydospore. In: Arai T (ed) Filamentous microorganisms: biomedical aspects. Japan Scientific Societies Press, Tokyo, pp 1–20
- Kendrick B, Watling R (1979) Mitospores in Basidiomycetes. In: Kendrick B (ed) The whole fungus. National Museum of Natural Sciences, Ottawa, pp 473–545
- Kobayashi H, Hatano K (2001) A morphological study of the mycorrhiza of *Entoloma clypeatum* f. *hybridum* on *Rosa multiflora*. *Mycoscience* 42:83–90
- Lefevre C, Müller W (1998) Description code 18. *Tricholoma magnivelare* (Peck) Redhead + *Pinus contorta* Dougl. var. *latifolia* Engelm. In: Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) Concise descriptions of North American ectomycorrhizae. Mycologue Publications and the Canada-BC Forest Resource Development Agreement, Pacific Forestry Centre, Victoria
- Simazono H (1979) Comparative studies on morphological characteristics of the colonies of *Tricholoma matsutake*, *T. fulvocastaneum* and *T. bakamatsutake* on agar media (in Japanese). *Trans Mycol Soc Jpn* 20:176–184
- Terashima Y, Tomiya K, Takahashi M, Iwai H (1993) Distribution and characteristics of shiros of *Tricholoma bakamatsutake* in a mixed forest of *Pasania edulis* and *Castanopsis cuspidata* var. *sieboldii*. *Trans Mycol Soc Jpn* 34:229–238
- Ulloa M, Hanlin RT (2000) Illustrated dictionary of mycology. APS, St. Paul, MN
- Yamada A, Maeda K, Ohmasa M (1999) Ectomycorrhiza formation of *Tricholoma matsutake* isolates on seedlings of *Pinus densiflora* in vitro. *Mycoscience* 40:455–463